The major goal of this project was to develop synthetic saponins as vaccine adjuvants to be used with GP antigen against Marburg virus.
Role: Principal Investigator

URC Grant Ashish K. Pathak (PI) 5/01/2007 – 4/30/2008
University Research Council (URC), Western Illinois University
Novel methods for efficient synthesis of oligosaccharides
The major goal of this project was cost effective synthesis of oligosaccharides using ionic liquid as reaction support.
Role: Principal Investigator

URC Grant Ashish K. Pathak (PI) 5/01/2006 – 4/30/2007
University Research Council (URC), Western Illinois University
Synthesis of hexasaccharide for antigen preparation in MTB vaccine
The major goal of this project was cost effective synthesis of hexasaccharide to be used as antigen in MTB vaccine.
Role: Principal Investigator

Ongoing Research Support for Ashish K. Pathak, Ph.D.

(b)(4)

1U19AI109680-01 Prof. R.J. Whitley (PI) 03/01/2014 – 02/28/2019
NIH/NIAID $1,046,665 (Core C)
Antiviral Drug Discovery and Development Center
The herein proposed Center of Excellence for Translational Research (CETR), which will be named the Antiviral Drug Discovery and Development Center (AD3C) has, at its center, the theme to develop new small molecule therapeutics for emerging and re-emerging viral infections. Translational research will focus on the inhibition of viral replication, especially viral polymerase.

Role: Medicinal Chemistry Core PI/Senior Medicinal Chemist

Pending Research Support for Ashish K. Pathak, Ph.D.

None

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Sheahan, Timothy Patrick

eRA COMMONS USER NAME (credential, e.g., agency login): (b)(6)

POSITION TITLE: Research Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of New Hampshire	B.S.	06/1999	Microbiology/Water Resources
University of North Carolina at Chapel Hill	Ph.D.	05/2008	Virology
The Rockefeller University	Postdoctoral	03/2013	Systems Virology

A. Personal Statement

I have the expertise and leadership to successfully carry out the proposed research of this project. I have a broad background in molecular virology, systems biology, vaccinology and animal models of viral pathogenesis. Through my graduate research, I gained extensive knowledge of coronavirus molecular biology, pathogenesis and evaluated multiple therapeutics aimed at blocking future emergence of zoonotic SARS coronavirus. I gained the time management and leadership skills required to execute grant-guided research during my postdoctoral F32 fellowship through NIAID. Lastly, due to my time in the pharmaceutical industry, I am well versed in the process of drug development and have an appreciation for the preclinical data needed to drive antiviral products forward in clinical development. Thus, my previous professional, scientific and organizational experience makes we well suited to continue antiviral drug discovery with this CETR grant.

B. Positions and Honors

Positions and Employment

1999-2001 Laboratory Technician, Harvard Gene Therapy Initiative, Harvard Medical School, Boston, MA.
2001-2003 Laboratory Technician, Tissue Engineering Laboratory of Joseph Vacanti. Massachusetts General Hospital, Boston, MA.
2003-2008 Graduate Student, Laboratory of Ralph S. Baric, University of North Carolina, Chapel Hill, NC.
2008-2013 Postdoctoral Fellow, Laboratory of Charles M. Rice, The Rockefeller University, NY, NY.
2013-2015 Investigator, Antiviral Discovery Performance Unit, GlaxoSmithKline, RTP, NC.
2015- Research Assistant Professor, Department of Epidemiology, University of North Carolina, Chapel Hill, NC.

Other Experience and Professional Memberships

2002- Member, American Society for Microbiology
2007- Member, American Society for Virology

Honors

1998 Gordon Byers Scholarship for an Outstanding Water Resources Student.
2002 Partners in Excellence Award, Massachusetts General Hospital.
2009 Ruth L. Kirschstein National Research Service Award (Postdoctoral Fellowship).
2015 Third Place Regional GSK Beautiful Biology Award. *"In vivo imaging: A new platform to accelerate drug discovery at the host/pathogen interface".*
2015 Second Place Global GSK Beautiful Biology Award. *"In vivo imaging: A new platform to accelerate drug discovery at the host/pathogen interface".*

C. Contributing to Science

1. My early work directly addressed the molecular evolution of animal associated coronavirus and how this zoonotic virus evolved to become epidemic SARS coronavirus in humans. Due to the recent emergence of MERS coronavirus, the theme of my earlier work continues to be germane to global public health.
    a. Roberts A, Deming D, Paddock CD, Cheng A, Yount B, Vogel L, Herman BD, **Sheahan T**, Heise M, Genrich GL, Zaki SR, Baric R, Subbarao K. A mouse-adapted SARS-coronavirus causes disease and mortality in BALB/c mice. *PLoS Pathogens.* 2007 Jan;3(1):e5.
    b. **Sheahan T**, Rockx B, Donaldson E, Sims A, Pickles R, Corti D, Baric R. Mechanisms of Zoonotic SARS-CoV Host Range Expansion in Human Airway Epithelium. *Journal of Virology.* 2008 Mar;82(5):2274-85.
    c. **Sheahan T**, Rockx B, Donaldson E, Corti D, Baric R. Pathways of Cross Species Transmission of Synthetically Reconstructed Zoonotic SARS-CoV. *Journal of Virology.* 2008 Sep;82(17):8721-32.
    d. **Sheahan T**, Morrison T, Funkhouser W, Akira S, Heise M, Baric R. MyD88 is required for protection from lethal infection with a mouse adapted SARS-CoV. *PLoS Pathogens.* 2008 Dec;4(12):e1000240.
2. The evaluation of and the development of therapeutics has consistently been a focus of my research since the beginning my career. Developing vaccines and monoclonal antibodies against viruses that exist as a genetically related but distinct quasispecies is very challenging due to the diversity of the target pathogen. Thus, vaccines and antibodies must be broadly cross-reactive to be effective. Using state of the art coronavirus and hepatitis C virus in vitro and in vivo models, the studies below demonstrate that unique tools and stringent evaluation are needed to drive the development of antiviral therapies with maximal utility to protect global public health.
    a. Deming D, **Sheahan T**, Heise M, Yount B, Davis N, Sims A, Suthar M, Harkema J, Whitmore A, Pickles R, West A, Donaldson E, Curtis K, Johnston R, Baric R. Vaccine efficacy in senescent mice challenged with recombinant SARS-CoV bearing epidemic and zoonotic spike variants. *PLoS Medicine.* 2006 Dec;3(12):e525.
    b. **Sheahan T**, Whitmore A, Rogers K, Ferris M, Rockx B, Funkhouser W, Donaldson E, Gralinski L, Collier M, Heise M, Davis N, Johnston R, Baric R. Successful Vaccination Strategies that Protect Aged Mice from Lethal Influenza and Lethal Heterologous SARS-CoV Challenge. *Journal of Virology.* 2011 Jan;85(1):217-30.
    c. Meuleman P, Catanese MT, Verhoye L, Desombere I, Farhoudi A, Jones CT, **Sheahan T**, Grzyb K, Cortese R, Rice CM, Leroux-Roels G, Nicosia A. A human monoclonal antibody targeting SR-BI precludes hepatitis C virus infection and viral spread in vitro and in vivo. *Hepatology.* 2012 Feb;55(2):364-72.
    d. **Sheahan TP**, Imanaka N, Marukian S, Dorner M, Liu P, Ploss A, Rice CM. Interferon Lambda Alleles Predict Innate Antiviral Immune Responses and Hepatitis C Virus Permissiveness. *Cell Host and Microbe.* 2014 Feb 12;15(2):190-202
3. The process of antiviral discovery and preclinical development of antiviral therapies is often not the focus of academic research. While working at GlaxoSmithKline, I gained important working knowledge of antiviral drug discovery and development. Leading a collaborative project between industry and academia, I gained important leadership and organizational skills and these experiences will help guide the proposed research program.
    a. Wood ER, Bledsoe R, Chai J, Daka P, Deng H, Ding Y, Harris-Gurley S, Kryn LH, Nartey E, Nichols J, Nolte RT, Prabhu N, Rise C, **Sheahan T**, Shotwell JB, Smith D, Tai V, Taylor JD, Tomberlin G, Wang L, Wisely B, You S, Xia B, Dickson H. The Role of Phosphodiesterase 12 (PDE12) as a Negative Regulator of the Innate Immune Response and the Discovery of Antiviral Inhibitors. *Journal of Biological Chemistry.* 2015 Jun 8.

b. Tran V, Poole DS, Jeffery JJ, **Sheahan TP**, Creech D, Yevtodiyenko A, Peat AJ, Francis KP, You S, Mehle A. Multi-Modal Imaging with a Toolbox of Influenza A Reporter Viruses. Viruses. 2015 Oct 13;7(10):5319-27.

Complete List of Published Work in NCBI MyBibliography:
http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/40066608/?sort=date&direction=descending

D. Research Support
Ongoing Research Support

ACTIVE:

U19AI107810 (PI: Baric) 06/21/13-05/31/18 NIH/NIAID
Characterization of novel genes encoded by RNA and DNA viruses
Using highly pathogenic human respiratory and systemic viruses, which cause acute and chronic life-threatening disease outcomes, we test the hypothesis that RNA and DNA viruses encode common and unique mechanisms to manipulate virus replication efficiency and host responses to determine severe disease outcomes.
Role: Investigator

U19 AI 109680 CETR (PI: Whitley) 03/01/14-02/28/19 UAB/NIH/NIAID
Antiviral Drug Discovery and Development Center
The specific aims of the proposal will identify small molecule inhibitors of CoV fidelity and RNA capping, define their mechanism of action, and determine their efficacy against SARS-CoV and across CoV families using in vivo mouse models of acute and persistent CoV disease.
Role: Investigator

U19 AI109761 CETR (PI: Lipkin) 03/01/14-02/28/19 Columbia/NIH/NIAID
Diagnostic and Prognostic Biomarkers for Viral Severe Lung Disease
The overall goal of this program is to develop new platform technologies that use functional genomics as diagnostic and prognostic indicators of severe end stage lung disease following virus infection of the lung.
Role: Investigator

R01 (PI: Baric) 04/01/15-03/31/20 NIH/NIAID
Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis
The overall goal is to build a comprehensive understanding of the molecular mechanisms guiding group 2c CoV receptor recognition, entry and pathogenesis.

Completed Research Support
F32 AI 084448 Sheahan (PI) 9/1/2009 – 9/31/2012
Hepatitis C virus host interactions in micropatterned hepatocyte co-cultures. The goal of this study was to develop technology facilitating the transcriptional profiling of HCV infected primary human hepatocytes.
Role: PI

OTHER SUPPORT

SHEAHAN, TIMOTHY

ACTIVE:

U19AI107810 (PI: Baric) 06/21/13-05/31/18 (b)(4)
NIH/NIAID $2,027,645
Characterization of novel genes encoded by RNA and DNA viruses
Using highly pathogenic human respiratory and systemic viruses, which cause acute and chronic life-threatening disease outcomes, we test the hypothesis that RNA and DNA viruses encode common and unique mechanisms to manipulate virus replication efficiency and host responses to determine severe disease outcomes.
Role: Investigator

U19 AI 109680 CETR (PI: Whitley) 03/01/14-02/28/19 (b)(4)
UAB/NIH/NIAID $1,611,425
Antiviral Drug Discovery and Development Center
The specific aims of the proposal will identify small molecule inhibitors of CoV fidelity and RNA capping, define their mechanism of action, and determine their efficacy against SARS-CoV and across CoV families using in vivo mouse models of acute and persistent CoV disease.
Role: Investigator

U19 AI109761 CETR (PI: Lipkin) 03/01/14-02/28/19 (b)(4)
Columbia/NIH/NIAID $2,999,060
Diagnostic and Prognostic Biomarkers for Viral Severe Lung Disease
The overall goal of this program is to develop new platform technologies that use functional genomics as diagnostic and prognostic indicators of severe end stage lung disease following virus infection of the lung.
Role: Investigator

R01 (PI: Baric) 04/01/15-03/31/20 (b)(4)
NIH $3,683,050
Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis
The overall goal is to build a comprehensive understanding of the molecular mechanisms guiding group 2c CoV receptor recognition, entry and pathogenesis.

OVERLAP:

If other awards are made, Dr. Sheahan will reduce his percent effort accordingly.

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Withheld pursuant to exemption

(b)(6) ; (b)(3):7 U.S.C. § 8401

of the Freedom of Information and Privacy Act

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Withheld pursuant to exemption

(b)(6) ; (b)(3):7 U.S.C. § 8401

of the Freedom of Information and Privacy Act

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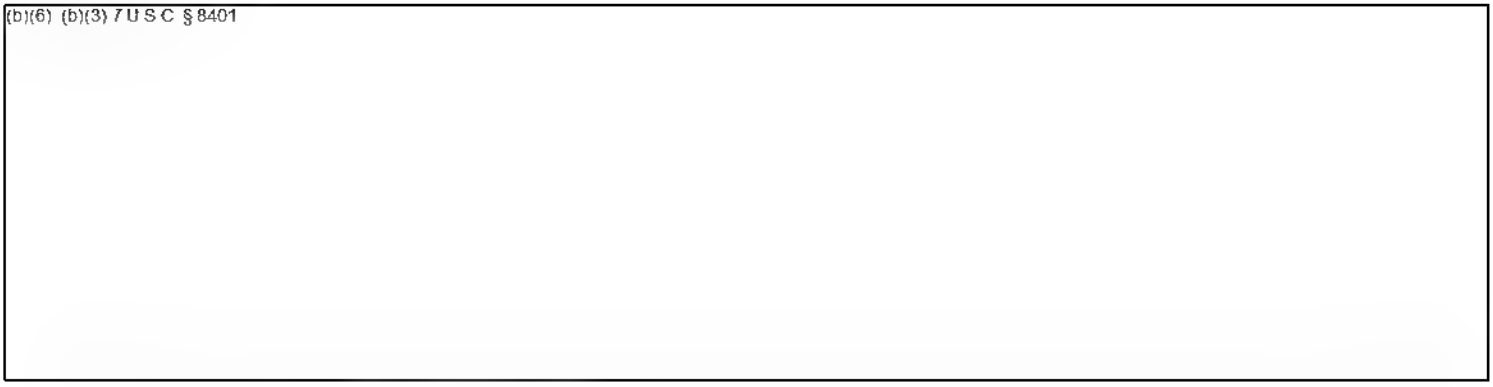
Withheld pursuant to exemption

(b)(6) ; (b)(3):7 U.S.C. § 8401

of the Freedom of Information and Privacy Act


(b)(6) (b)(3) 7 U S C § 8401

D.2.b (New Version Umbrella new key personnel pdf)

(b)(6) (b)(3) 7 U S C § 8401

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Withheld pursuant to exemption

(b)(6) , (b)(3) 7 U.S.C. § 8401

of the Freedom of Information and Privacy Act

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of the Freedom of Information and Privacy Act

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of the Freedom of Information and Privacy Act

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Withheld pursuant to exemption

(b)(6) , (b)(3) 7 U.S.C. § 8401

of the Freedom of Information and Privacy Act

Following are updated documents for the Other Support of Senior/Key Personnel involved in the U19; they start with Dr. Whitley's document, the rest is in alphabetical order of their last name:

- Whitley, Richard J.
- Augelli-Szafran, Corinne
- Baric, Ralph
- DeFilippis, Victor
- (b)(6), (b)(3) 7 U S C § 8401
- Diamond, Michael
- Everts, Maaike
- (b)(6), (b)(3) 7 U S C § 8401
- (b)(6), (b)(3) 7 U S C § 8401
- Hirsch, Alec
- Nelson, Jay
- Prichard, Mark
- (b)(6) (b)(3) 7 U S C § 8401
-
- Streblow, Dan
- Suto, Mark

For New and Renewal Applications (PHS 398) – DO NOT SUBMIT UNLESS REQUESTED

PHS 398 OTHER SUPPORT

WHITLEY, R.J.

ACTIVE

HHSN272201100034C (Whitley, Kimberlin Co PI) 9/28/11-9/27/17 (b)(4)
HHS-NIH-NIAID $3,385,690
An adaptive sequential study evaluating prevention of neonatal HSV: Detection of maternal shedding at delivery followed by preemptive antiviral therapy in exposed neonates.
In this project, a multi-institutional team of investigators, known as the Collaborative Antiviral Study Group (CASG), will validate the GeneXpert® HSV polymerase chain reaction (PCR) system by comparing it against standard quantitative PCR and routine viral culture.

HHSN272201100035C (Whitley, Kimberlin Co PI) 9/28/11-9/27/17 (b)(4)
HHS-NIH-NIAID $2,065,894
A Phase II 6 Weeks oral Valganciclovir vs Placebo in infants with Congenital CMV infection and hearing loss.
In this project, the CASG will identify infants and toddlers with SNHL and then will test these patients' DBS obtained during the neonatal period for CMV DNA by PCR.

HHSN272201100036C (Whitley, Gnann Co PI) 9/28/11-9/27/17 (b)(4)
HHS-NIH-NIAID $2,188,470
Natural History of Infection Caused by BK Virus (and other Opportunistic Viral Pathogens) in Renal and Renal-Pancreas Transplant Recipients
The primary objective of the study is to determine the safety, tolerability and optimal dose of CMX001 in kidney transplant patients with BKV viremia.

HHSN272201100037C (Whitley, Kimberlin Co PI) 9/28/11-9/27/17 (b)(4)
HHS-NIH-NIAID $1,779,753
A PK/PD and Resistance Evaluation of Intravenous Ganciclovir in Premature Infants
In this project, a multi-institutional group of investigators, known as the Collaborative Antiviral Study Group (CASG), will enroll premature subjects who are being treated clinically with intravenous ganciclovir for postnatally or congenitally acquired CMV disease.

HHSN272201100038C (Whitley, Kimberlin Co PI) 9/28/11-9/27/19 (b)(4)
HHS-NIH-NIAID $1,967,277
Adaptive study of CMX-001 in infants with Neonatal Herpes Simplex Virus (HSV)
In this project, a multi-institutional team of investigators, known as the Collaborative Antiviral Study Group (CASG), will define the pharmacokinetics (PK) and concentration response relationship of CMX001 in neonates with HSV CNS disease.

1U19AI109680-02 (Whitley, PI) 3/1/14-2/28/19 (b)(4)
HHS-NIH- NIAID $1,210,504 Admin core; $780,179-Proj 4
Center for Antiviral Drug Discovery and Development-UAB
Role: Administrative Core: PI, and Project Director; Project 4.0 – Influenza: Co-PI
Consortium which focuses on development of antiviral therapeutics for four major categories of emerging infectious diseases including Flaviviruses, Corona viruses, Alphaviruses and Influenza

U54TR001368-01 (Kimberly) 9/01/15 – 8/31/20 (b)(4)
NIH/NCATS $6,324,075 (UL1, KL2, TL1)
UAB Center for Clinical and Translational Science (CCTS)
The UAB CCTS will enhance human health by driving scientific discovery and dialogue across the bench, bedside and community continuum. The CCTS supports this overall mission in a highly integrative network of relationships. Five strategic priorities are: 1) enhancing research infrastructure; 2) promoting investigator education, training and development; 3) accelerating discovery across the T1 interface; 4) expanding value-added partnerships; and 5) building sustainability.
Role: Co-Investigator, Project Leader

UM1 AR065705 (Curtis, Winthrop, MPI) 9/01/14 – 8/31/19 (b)(4)
NIH/NIAMS $437,212 Annual Direct Costs
Safety and Effectiveness of Live Zoster Vaccine in Anti- TNF Users (VERVE)
The Varicella zostER VaccinE (VERVE) trial is a randomized, double-blind, placebo-controlled large pragmatic trial to evaluate the immunogenicity, safety, and longer-term effectiveness of the live HZ vaccine in arthritis patients receiving anti-TNF therapy
Role: Advisor

PENDING

None

COMPLETED

N01-AI-30025 (Whitley, PI) 8/1/03-12/01/13 (b)(4)
NIH-NIAID $3,719,919
Clinical Trials for Antiviral Therapies
This contract serves to facilitate the development of promising therapies for treating severe, acute, and chronic human viral diseases that are deemed medically and scientifically important by the NIH-NIAID. This program will facilitate advances in clinical antiviral therapy by rigorously evaluating the efficacy and safety of new therapeutic regimens for serious viral diseases in adult and pediatric patient populations.

3 U54 A1057157 (Sparling-UNC PI) 3/01/09–2/28/14 (b)(4)
NIH $31,000
SERCEB Southeast Regional Centers for Excellence for Biodefense
This grant is jointly submitted by investigators in the Southeastern United States and researches new ways to contribute to the national effort in biodefense, as well as to study emerging infectious diseases that threaten both our country and our world.
Role: Co-Investigator.

2K12HD043397-09 (Stagno, PI) 3/10/08-11/30/13 (b)(4)
NIH $349,821
Pediatric Physician Scientist in Translational Molecular Biology
The goal is to enhance the mentored research experience with a foundation of research techniques and approaches. Role: Co-Investigator.

1U54 RR024376-05 (Kimberly, PI) 7/1/08-4/30/14 (b)(4)
NIH $232,220
UAB Center for Clinical and Translational Science (CCTS)

The UAB Center for Clinical and Translational Science (CCTS) will transform the UAB environment by building productive and efficient interdisciplinary research teams through educational ingenuity, regulatory reorganization, resource coordination, and methodological innovation. Its mission is to develop a transformative infrastructure that spans the spectrum from pre-clinical research to bench-to-bedside translation (T1 research) to community implementation (T2 research).
Role: Co-Investigator, Project Leader

1UL1RR025777-01(Kimberly, PI) 05/01/14-4/30/15 (b)(4)
NIH $80,109
UAB Center for Clinical and Translational Science (CCTS) supplement
The UAB Center for Clinical and Translational Science (CCTS) Drug Discovery project continuation which seeks to provide program for academic drug development in fulfillment of the overall mission to develop a transformative infrastructure that spans the spectrum from pre-clinical research to bench-to-bedside translation (T1 research) to community implementation (T2 research).
Role: Co-Investigator, Project Leader

5P01-CA 071933-15 (Whitley, PI) 7/1/09-6/30/15 (b)(4)
NIH-NCI $218,869
Engineered HSV for the Treatment of Malignant Glioma
The long-term objective of this program project grant, in collaboration with Dr. Bernard Roizman at the University of Chicago, was the design and testing of novel recombinant herpes simplex viruses as vectors of noxious genes for the selective destruction of human glioma cells.

NAME OF INDIVIDUAL: CORINNE AUGELLI-SZAFRAN

ACTIVE/PENDING

Project Number (Principal Investigator) Source Title of Project *(or Subproject)* The major goals of this project are...	Dates of Approved/Proposed Project Annual Direct Costs	Person Months (Cal/Academic/ Summer)

OVERLAP *(summarized for each individual)*

ACTIVE –

BAO# 28XS124 TO#8 (Suto, PI) 10/01/2013 03/31/2016 (b)(4)
SAIC (NCI) $2,749,805 total
cost

Chemical Biology Consortium — Collaborative Drug Discovery Partnership with NCI. The goals of this NCI program are to provide a comprehensive research framework leading to the development of new anticancer drugs. The program focuses on the identification of new molecular targets, and the rapid development of these from medicinal chemistry through final drug development. As a Comprehensive Research Center within the network, Southern Research will implement a wide variety of cell-based and biochemical assays for the development of novel drugs, and to develop second-generation lead compounds and drugs based on the identified screening hits. Task Order #8 – Administrative Support

1U19AI109680, Whitley (PI) 03/01/2015 – 02/28/2019 (b)(4)
NIAID $730772

Antiviral Drug Discovery and Development Center. The goals of this NIAID program are:
Development of antiviral drugs for the treatment of emerging and reemerging infections. Specifically, the focus will be on flaviviruses, alphaviruses, corona viruses and influenza. The goal is to identify compounds working through mechanisms that affect viral replication and develop these leads in a translational manner to new human therapeutics. As described above, each of the projects is focused on a viral family deemed critical to NIAID's focus on Emerging and Re-emerging Infectious Diseases related to biodefense. Role: Co-Investigator

HHSN272201400010I, Ptak (PM) 08/01/2014 – 7/31/2021 (b)(4)
NIAID $3,006,479 (total cost)

In Vitro Testing Resource for HIV Therapeutics and Topical Microbicides.
NIAID service resource for testing of anti-HIV therapeutics and topical microbicides. Contract includes assay development, high throughput screening, and chemistry initiatives for identifying and developing novel inhibitors of HIV-1, as well as cataloguing of HIV protein interactions reported in the scientific literature. Role: Co-Investigator

HHSN272201400010I, Ptak (PM) 08/01/2015 – 7/31/2017 (b)(4)
NIAID Task Order16 $152,117

In Vitro Testing Resource for HIV Therapeutics and Topical Microbicides.
NIAID service resource for testing of anti-HIV therapeutics and topical microbicides. Contract includes assay development, high throughput screening, and chemistry initiatives for identifying and developing novel inhibitors of HIV-1, as well as cataloguing of HIV protein interactions reported in the scientific literature. Role: Co-Investigator.

HHSN272201400010I, Ptak (PM) 09/01/2014 – 7/31/2016 (b)(4)

NIAID Task Order 9 $284,267

In Vitro Testing Resource for HIV Therapeutics and Topical Microbicides.
NIAID service resource for testing of anti-HIV therapeutics and topical microbicides. Contract includes assay development, high throughput screening, and chemistry initiatives for identifying and developing novel inhibitors of HIV-1, as well as cataloguing of HIV protein interactions reported in the scientific literature.
Role: Co-Investigator

UAB, Robinson (PI) 9/01/2015 – 08/31/2016 (b)(4)
(b)(4) $26,034

Goal: Development of Inhibitors of the Tau-Fyn Interaction for the Treatment of Alzheimer's disease. This project aims to develop inhibitors of the Tau–Fyn interaction for treatment of Alzheimer's disease and represents a new therapeutic strategy for the disease.
Role: Co- Investigator

PENDING - None

OVERLAP - None

BARIC, RALPH S.

ACTIVE:

U19 AI 107810 (PI: Baric) 06/21/13-05/31/18 (b)(4)
NIH/NIAID $1,558,483
Characterization of novel genes encoded by RNA and DNA viruses
Using highly pathogenic human respiratory and systemic viruses which cause acute and chronic life-threatening disease outcomes, we test the hypothesis that RNA and DNA viruses encode common and unique mechanisms to manipulate virus replication efficiency and host responses to determine severe disease outcomes.

U19-AI100625 (PI: Baric/Heise-MPI) 08/05/12-07/31/17 (b)(4)
NIH/NIAID $3,152,632
Systems Immunogenetics of Biodefense Pathogens in the Collaborative Cross
Specific Aims: In this proposal, we are utilizing the Collaborative Cross (CC), a novel panel of reproducible, recombinant inbred (RI) mouse lines to identify genes and gene interactions which regulate the induction, kinetics, and magnitude of the innate, inflammatory and adaptive arms of the immune response following virus infection. Specifically, we will develop novel modeling algorithms to predict and validate the causal relationships between natural genetic variation and host signaling networks, immune cell recruitment, and immune function.

00008956 (PI: De Silva) 07/29/15-06/30/16 (b)(4)
UCB/NIH $275,000
Protective immunity following dengue virus natural infections and vaccination
We will perform studies to characterize the B-cell/ antibody (responses in people who receive dengue live attenuated virus vaccines (DLAV).
Role: Co-Investigator

R01 AI 107731 (PI: De Silva) 08/05/13-07/31/17 (b)(4)
NIH/NIAID $378,675
Molecular Basis of Dengue Virus Neutralization by Human Antibodies
These studies proposed here are directly relevant to developing simple assays to predict the performance of the leading dengue vaccine candidates and also for developing the next generation of safe and effective dengue vaccines.
Role: Co-Investigator

R01 AI108197 (MPI: Denison/Baric) 08/01/13-07/31/17 (b)(4)
Vanderbilt University/NIH/NIAID $450,129
Determinants of Coronavirus Fidelity in Replication and Pathogenesis
Experiments in this aim will test the hypothesis nsp1 functions in maintaining high replication fidelity and viral RNA synthesis are coupled and that targeted engineered mutations across nsp14 alter: a) RNA fidelity outcomes; b) sensitivity nucleoside mutagens, terminators and polymerase inhibitors; c) the kinetics and magnitude of positive, negative, genomic and subgenomic RNA synthesis; and d) RNA recombination frequencies.

U19-AI106772-01 (PI: Kawaoka) 06/01/13-05/31/16 (b)(4)
Univ of Wisconsin/NIH $780,782
MERS-CoV Supplement for OMICs Proposal
The proposed studies will provide a more detailed look at the intracellular environment by taking "snapshots" of the lipids, metabolytes, and proteins present during viral infection time courses. These assays will allow us to determine the innate immune response occurring immediately following virus infection and to determine how the virus and cell interact over a 72 hour window.
Role: Investigator

HHSN272201000019I-HHSN27200003 (PI: Baric) 09/30/11-11/14/15 (b)(4)
MSSM/NIH $481,223
MERS-CoV Mouse Model for Vaccine and Therapeutic Testing (Task Order A57)
Specific Aims: Use generation of transgenic mice and modifications to the MERS-CoV genome to identify a mouse model for MERS-CoV that recapitulates human disease phenotypes for evaluating vaccine platforms and therapeutics.

U19 AI 109680 CETR (PI: Whitley) 03/01/14-02/28/19 (b)(4)
UAB/NIH/NIAID $314,437
Antiviral Drug Discovery and Development Center
The specific aims of the proposal will identify small molecule inhibitors of CoV fidelity and RNA capping, define their mechanism of action, and determine their efficacy against SARS-CoV and across CoV families using in vivo mouse models of acute and persistent CoV disease.
Role: Co-Investigator

U19 AI109761 CETR (PI: Lipkin) 03/01/14-02/28/19 (b)(4)
Columbia/NIH/NIAID $2,999,060
Diagnostic and Prognostic Biomarkers for Viral Severe Lung Disease
The overall goal of this program is to develop new platform technologies that use functional genomics as diagnostic and prognostic indicators of severe end stage lung disease following virus infection of the lung.
Role: Project Leader, Consortium PI

246823 (PI: Baric) 01/27/15-09/16/16 (b)(4)
PNNL/DHS $205,569
The Generation of Predictive Models of Viral Pathogenesis
Using advances in transcriptomics, proteomics, and metabolomics, we will identify changes in the virus-host interaction expression networks associated with DENV infection of Aedes aegypti cells or human immune cells in vitro, the latter model after natural receptor-mediated or after ADE mediated entry processes.

R56 AI106006 (PI: Baric) 09/01/14-08/31/16 (b)(4)
NIAID/NIH $597,745
Mechanisms of Norovirus Protective Immunity
The goal of this grant is to prevent future norovirus outbreaks. We propose to identify molecular markers for long-term protective immunity and characterize the breadth of the protective antibody response after vaccination. Our studies will identify key norovirus neutralizing epitopes which mediate type specific and broadly cross reactive short and long-term protective immunity, develop robust platforms for discriminating between short and long-term memory B cell response following human vaccination and inform second generation norovirus vaccine design as certain strains evolve quickly.

Not assigned (PI: deSilva) 11/05/14-09/30/17 (b)(4)
(b)(4) $726,498
The dengue human infection model: Defining correlates of protection and advancing vaccine development
The goal of these studies conducted by the Baric laboratory are to use recombinant dengue viruses encoding multiple homotypic neutralizing sites from multiple strains, as well as a collection of null mutants, to characterize the homotypic immune response elicited in humans following natural infection and after challenge in (b)(4) DENV tetravalent vaccinated individuals. This grant has been funded by (b)(4)
Role: Co-Investigator

R01 AI110700 (PI: Baric) 04/01/15-03/31/20 (b)(4)
NIH/NIAID $617,400
Mechanisms of MERS-CoV Entry, Cross-species Transmission and Pathogenesis
The overall goal is to build a comprehensive understanding of the molecular mechanisms guiding group 2c CoV receptor recognition, entry and pathogenesis.

60045042 (PI: Baric) 02/01/15-01/31/18 (b)(4)
Ohio State/USDA ARS $21,428

Molecular attenuation mechanisms of porcine epidemic diarrhea virus in pigs

Reverse genetic strategies are used to construct a panel of live attenuated porcine epidemic diarrhea recombinant viruses for in vivo pathogenesis studies and in vitro biological characterization. We test rationale vaccine strategies to protect new born piglets against this devastating porcine epidemic virus.

PENDING

OTHER SUPPORT FOR ALL KEY PERSONNEL – OREGON HEALTH & SCIENCE UNIVERSITY

DEFILIPPIS, V

ACTIVE

HHSN272201400055C (Nelson) 9/30/2014 – 9/29/2019 (b)(4)
Adjuvant Discovery Program $1,642,310
Targeting IRFs for Immune Adjuvant Enhancement of Vaccine Immunogenicity
The overall goal of this contract is to use a high-throughput screening (HTS) program to identify and develop small molecule adjuvants that activate interferon-regulatory factors (IRFs) that enhance protective immunity for vaccines to NIAID Category A-C viruses.
Role: Co-Investigator

(b)(4) (Picker) 7/28/2014 – 8/31/2019 (b)(4)
$6,144,126
Development of Attenuated CMV Vectors for an HIV/AIDS Vaccine
The overall goal of this project is the development of an HCMV vector-based HIV/AIDS vaccine (composed of one or more HIV insert-expressing HCMV vectors) that is optimized for safety, efficacy and manufacturability.
Role: Project Leader

(THIS AWARD; Project 3B)
5 U19 AI109680-02 (Whitley) 3/01/2014 – 2/28/2019 (b)(4)
NIH/NIAID $299,955
Antiviral Drug Discovery and Development Center
Project 3B: Novel Therapeutic Strategies Targeting Re-emerging Alphaviruses
The main goal of this project is to develop novel nucleoside and nucleotide inhibitors directed against Alphaviruses including Chikungunya virus and Venezuelan Equine Encephalitis virus.
Role: Project 3B Co-Investigator

5 R01 AI059457-10 (Früh) 7/01/2004 – 11/30/2015 (b)(4)
NIH/NIAID $270,051
Evasion of Antigen Presentation by Rhesus Cytomegalovirus
This grant focuses on immune evasion characteristics of CMV that hamper development of a HCMV vaccine, in particular, characterization of the biology of US2-11-related MHC I evasion functions in the genome of RhCMV.
Role: Staff Scientist

Departmental Support (b)(4)
Vaccine and Gene Therapy Institute

INACTIVE

5 U54 AI081680-05 (Nelson) 3/01/2011 – 2/28/2015 0.0 calendar
NIH/NIAID (no cost extension)
Pacific NorthWest Regional Center of Excellence Developmental Research Project
Role of cytokines in Chikungunya virus-associated disease
The goal of this project is an understanding of the contribution of these host-virus interactions to CHIKV-associated disease. CHIKV is a re-emerging arthritogenic mosquito-borne RNA Alphavirus. Infection results in serum induction of multiple proinflammatory cytokines including type I interferons (IFN).
Role: Developmental Research Project (DP005) PI

OVERLAP No overlap

OTHER SUPPORT

DIAMOND, MICHAEL

ACTIVE

1006666 (Diamond) 07/01/07-06/30/16
(b)(4) $150,000 (NCE) (b)(4)
Role: PI

Epitope-Based Immunogens and Diagnostics for Dengue Virus.
This grant focuses on the development of epitope-based diagnostics against Dengue Virus.

R01A1089591 (Diamond) 06/01/10-05/31/16
NIH/NIAID $374,265 (no cost extension) (b)(4)
Role: PI

Antibody-Based Therapy of Chikungunya Virus
This grant studies the basic structural and molecular biology of antibody neutralization of Chikungunya virus as a foundation for developing novel protective immunotherapeutics against this emerging alphavirus.

R01AI098715 (Rico-Hesse) 05/01/12 – 05/31/2016
NIH/NIAID $65,837 (b)(4)
Role: Co-Investigator

Therapy of Dengue with Modified Antibodies in Humanized Mice
The foundation of this proposal is the existing collaboration between an academic (Diamond) laboratory with broad expertise in flavivirus pathogenesis and antibody neutralization, and a company (MacroGenics) with skill in humanization of mouse antibodies as therapeutic agents.
These collaborators have produced humanized mAbs against flaviviruses with optimized effector function and no possibility of antibody-dependent enhancement of infection. The Rico-Hesse laboratory brings experience in dengue virus pathogenesis, with emphasis on the use of wild-type viruses to test determinants of virulence and transmission. Dr. Rico-Hesse serves as the P.I. in this collaboration because most of the experiments described here will involve the use of the mouse model of disease developed in her laboratory. Thus, we propose an experienced collaborative group to test novel dengue virus immunotherapy in a more relevant small animal model of disease.

N01AI00017 (Nikolich) 05/16/2011-05/15/2016
NIH/NIAID $170,770 (b)(4)
Role: Co-Investigator

Immunological Basis of Age Related Vulnerability in Biodefense and Emerging Infections
Several emerging pathogens and bioterrorism agents, including class B agents West Nile virus (WNV) and L. monocytogenes and class C agent influenza virus, are especially deadly to older adults, for reasons incompletely understood at the present. The goal of this project is to elucidate basic mechanisms that lead to increased age-related vulnerability to infection in humans by using complementary mouse and human models.

R01AI101400 (Lemke) 08/21/12 – 07/31/16
NIH/NIAID $94,777 (b)(4)
Role: Co-Investigator

TAM Receptors and Flavivirus Infection
This grant studies how TAM receptors (Axl, Mer, and Tyro3) contribute to flavivirus pathogenesis. These molecules are now believed to enhance infection of West Nile and Dengue virus. Our lab will perform in vivo experiments in KO mice to determine the exact contribution of these molecules to pathogenesis.

R01AI098723 (Slifka) 05/01/2012-04/30/2017
NIH/NIAID $108,749 (b)(4)
Role: Co-Investigator

Development of an H202-Inactivated Dengue Virus Vaccine.
The role of the Diamond laboratory in this project will be to characterize the breadth and magnitude of the neutralizing antibody response against all genotypes of the four DENV serotypes. Neutralization assays will be performed in multiple cell types (macrophages, fibroblasts) using wild type and variant viruses and reporter

virus particles. Epitope-based diagnostics will be generated and used to map the type of neutralizing antibodies and whether they function in a type-specific manner. Additional analysis will include isotype analysis and avidity characterization. These studies will comprehensively establish the functional quality of antibodies generated by the candidate vaccine.

R33AI101329 (Chen) 06/01/12-05/31/17
NIH/NIAID $144,106
Role: Co-Investigator
(b)(4)

Bi-Functional Antibodies with Targeted CNS Delivery Against West Nile Virus
The Diamond laboratory will characterize the antiviral and immunological activity of different cell culture and plant derived bi-functional antibodies using mouse TfR-specific hE16 bifunctional antibody as a proof-of-principle in a mouse model of WNV infection. Our lab will test the hypothesis that TfR-Bif can achieve higher levels in the CNS and extends the window of treatment against WNV encephalitis.

R01AI104972 (Diamond) 04/01/13-03/31/18
NIH/NIAID $278,755
Role: PI
(b)(4)

ISG Control of Flavivirus Infection
The continual outbreaks of flavivirus disease highlight a need for an expanded understanding of mechanisms of immune control. Insight into the cell-intrinsic immune processes that restrict flavivirus infection is essential for developing novel strategies to contain disease. The studies in this collaborative and inter-disciplinary project between the Diamond and Chanda laboratories will use genetic screens to identify novel interferon stimulated genes (ISG) that modulate flavivirus infection in specific cell types ex vivo and in vivo.

U19AI106772 (Kawaoka) 07/01/13-06/30/18
NIH/NIAID $304,060
Role: Co-Investigator
(b)(4)

Modeling Host Responses to Understand Severe Human Virus Infections
Research in this program project grant will depend on unique cell and tissue reagents generated by the Diamond laboratory at Washington University. These reagents will be used to perform global transcriptome, proteomics, metabolomics, and lipidomic analysis by the Project Core facilities. Target genes will be identified by network analysis and the Diamond laboratory will validate these using reverse genetic approaches. In the last Aim, new KO mice will be generated to assess the physiological relevance of target genes in restricting West Nile virus infection in vivo.

R01AI073755 (Diamond) 07/01/13-06/30/2018
NIH/NIAID $128,757
Role: PI
(b)(4)

Antibody Based Protection against Dengue Virus.
This is a collaborative research project with the goal of defining new states of DENV particle structure and determining how these interact with specific MAbs. This information will be translated into developing a novel DENV vaccine strategy that traps virions in states that preferentially elicit highly neutralizing Abs.

R01AI104002(Gale) 07/01/13-06/30/18
NIH/NIAID $212,915
Role: Co-Investigator
(b)(4)

Innate Immune Control of West Nile Virus
The Diamond laboratory will perform a series of interactive studies aimed at understanding the mechanistic contribution of RLR signaling, specific IFN-β defenses restricting neuronal cell tropism, and viral counter-measures in determining WNV infection outcome. We hypothesize that WNV infection outcome is regulated by virus/host interactions that modulate RLR signaling and specific IFN-β innate immune defenses in distinct cell types.

HHSN272201400018C(Fremont) 09/30/14-09/29/18
NIH/NIAID $275,067
(b)(4)

Role:Co-Investigator
B-Cell Epitope Mapping of Viral and Parasitic Antigenes
The primary goal is the delineation of epitopes recognized by potently neutralizing antibodies and establishing correlates of protection that can aid in the development of vaccines and therapeutics against human pathogens

U19AI109680 (Whitley) 03/01/14 – 02/28/19 (b)(4)
NIH/NIAID $135,276
Role: Co-Investigator
Antiviral Drug Discovery and Development Center
This proposal is designed to identify small molecule compounds with the potential to be developed as antiviral agents. The initial screen in this proposal will focus on two medically relevant flaviviruses: dengue viruses (DENV) and West Nile virus (WNV). An existing screening platform will be adapted to screen extensive libraries of nucleoside and nucleotide analogs, potentially compounds that have activity against multiple flaviviruses. If such broad-spectrum leads can be identified, their further development will be emphasized. Our component of this projet will focus on compounds that target the 2'-O-methyltransferase, which is required for the virus to evade the host innate immune response

U19AI083019(Gale) 05/01/14 – 04/30/19 (b)(4)
NIH/NIAID $220,741
Role: Co-Investigator
Center for the Study of Immune Mechanisms of Flavivirus Control
Project 2: CNS Innate Immune Control of Encephalitic Flaviviruses
The primary goal of Project 2 is to determine how innate immune responses impact on the entry, infection, and replication of encephalitic flaviviruses within the central nervous system (CNS). Our experiments will assess the virus and host interface that regulates the innate immune response and controls WNV pathogenesis in the central nervous system, which will reveal novel targets for therapeutic development to suppress flavivirus infection and minimize neuronal injury.

HDTRA1-15-1-0013 (Diamond) 12/15/14 – 12/14/19 (b)(4)
DTRA $243,164
Role: PI
A novel Inactivated trivalent vaccine to prevent infection by Venezuelan, Eastern, and Western equine encephalitis viruses
The goal of this project is to determine the potency of Ab neutralization, extent of cross-neutralization against VEEV, EEEV,and WEEV, and location of key neutralizing epitopes on newly generated panels of monoclonal antibodies (MAbs). This project will elucidate the immunology and biology of encephalitic alphaviruses and generate an effective counter-measure to protect American citizens and military personnel against infection.

R01AI114816 (Crowe) 02/01/15-01/31/20 (b)(4)
NIH $174,685
Role: Co-Investigator
Structural and functional basis of ultra-potent CHKV neutralization by human mAbs
A primary goal of this project is to define the molecular, genetic, immunologic, and structural characteristics of ultra-potent neutralizing human mAbs with broad activity against all genotypes of CHIKV. Additional goals include defining the mechanistic correlates of protection by these ultra-potent neutralizing mAbs. In these studies, we will elucidate how antiviral Abs with exceptional inhibitory activity exert their action in cell culture and in vivo.

P01AI106695 (Harris) 7/01/2015-6/30/20 (b)(4)
NIH $100,000
Protective Immunity Following Dengue Virus Natural Infections and Vaccination
Co-Investigator
The Diamond laboratory will perform the initial screens of virus specificity and neutralization potentialof the monoclonal antibodies generated from peripheral blood mononuclear cells (PBMCs) after natural DENV

infection or immunization with tetravalent vaccines (obtained from Core C). They will also generate the labeled viruses needed for the DENV-specific sorting of memory B cells by the Crowe laboratory for one of the methods to be used in Core B for generating MAbs for Projects 1 and 2.

PENDING

(b)(4)

(b)(4)

OVERLAP
None
If pending applications are funded, Dr. Diamond will adjust his effort accordingly so as not to exceed twelve calendar months of funding.

EVERTS, M.

ACTIVE

(b)(4)

1 U19 AI109680-02 (Whitley) 3/1/2014-02/28/2019
NIH/NIAID $173,102 Admin core
Antiviral Drug Discovery and Development Center (AD3C)
Role: Administrative Core: Co-I and Associate Administrative Director

The major goals of this project are to develop new therapeutics for antiviral infections, in particular for alphaviruses, flaviviruses, coronaviruses and influenza.

(b)(4)

U54TR001368-01 (Kimberly) 9/1/15-8/31/20
NIH/NCATS $6,324,075 (UL1, KL2, TL1)
UAB Center for Clinical and Translational Science (CCTS)
Role: Co-Investigator

The UAB CCTS will enhance human health by driving scientific discovery and dialogue across the bench, bedside and community continuum. The CCTS support this overall mission in a highly integrative network of relationships. Success in creating such an environment is dependent upon success in achieving five strategic priorities: 1) enhancing research infrastructure; 2) promoting investigator education, training and development; 3) accelerating discovery across the T1 interface; 4) expanding value-added partnerships; and 5) building sustainability.

PENDING
None

OVERLAP
None

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Withheld pursuant to exemption

(b)(4) (b)(6) (b)(3) 7 U.S.C. § 8401

of the Freedom of Information and Privacy Act

OTHER SUPPORT – OREGON HEALTH & SCIENCE UNIVERSITY

HIRSCH, A

ACTIVE

(b)(4)

BAA-NIAID-DAIT-HIHAI2010085 (Nikolich-Zugich PI) 5/16/2011 – 5/15/2016
Subcontract Y562709 on NIH Contract No. HHSN272201100017C
NIH/NIAID $195,051
Protective Immunity in Special Populations: Interface between Innate and Adaptive Immunity
The major goal of this subcontract is to determine the role of miRNAs on immune function in the context of aging.
Role: Co-Investigator

(THIS AWARD)

(b)(4)

5 U19 AI109680-02 (Whitley) 3/01/2014 – 2/28/2019
NIH/NIAID $274,539
Antiviral Drug Discovery and Development Center: Project 1: Identification and Development of Anti-Flavivirus Lead Drug Candidates
The overall goal of this proposal is to discover and characterize compounds with broad anti-flavivirus activity.
Role: Co-Investigator

(b)(4)

HHSN27201400055C (Nelson) 9/30/2014 – 9/29/2019
NIH/NIAID $1,642,310
Adjuvant Discovery Program
Targeting IRFs for Immune Adjuvant Enhancement of Vaccine Immunogenicity
The overall goal of this contract is to use a high-throughput screening (HTS) program to identify and develop small molecule adjuvants that activate interferon-regulatory factors (IRFs) that enhance protective immunity for vaccines to NIAID Category A-C viruses.
Role: Co-Investigator

INACTIVE

(b)(4)

5 R21 AI101282-02 (Hirsch) 6/01/2012 – 5/31/2015
NIH/NIAID $120,846 (no-cost extension)
Evaluation of Host miRNAs as Therapeutics against Encephalitogenic Flaviviruses
The overall goal of this proposal is to use cellular microRNAs (miRNAs) as potential targets of therapeutic intervention for the neurotropic flaviviruses West Nile virus (WNV) and Japanese encephalitis virus (JEV).
Role: PI

(b)(4)

5 U54 AI 081680-05 (Nelson) 4/20/2009 – 2/28/2015
NIH/NIAID $5,688,792 (no-cost extension)
Pacific Northwest Regional Center of Excellence – Developmental Project: The role of microRNAs in flavivirus replication (DP 006)
The overall goal of this proposal is to elucidate how the observed changes in miRNA expression affect WNV replication and pathogenesis as well as to extend this analysis to the dengue viruses, which are also members of the flavivirus family.
Role: PI of Developmental Project

(b)(4)
(b)(4) (Hirsch) 9/15/2012 – 7/10/2014
$113,200

(b)(4)

Development of a Thienopyradine Compound as an Anti-Dengue Virus Therapeutic
The overall goal of this proposal is to identify the compound with DENV-inhibitory activity in high-content replication assay.
Role: PI

OVERLAP – No overlap

OTHER SUPPORT – OREGON HEALTH & SCIENCE UNIVERSITY

NELSON, JA

ACTIVE

5 R01 AI021640-30 (Nelson) 12/01/1984 – 1/31/2018 (b)(4)
NIH/NIAID $299,774
Molecular Aspects of Cytomegalovirus Latency

The long-term goal of this project is to develop an understanding of the cellular and molecular mechanisms of human cytomegalovirus (HCMV) persistence in the host. This project will use HCMV miRNA mutants as well as miRNA inhibitory molecules in an in vitro CD34+ human progenitor cell system and a humanized mouse model to examine the role of the viral miRNAs in latency and reactivation.
Role: PI

HHSN272201400055C (Nelson) 9/30/2014 – 9/29/2019 (b)(4)
NIH/NIAID $1,642,310
Adjuvant Discovery Program
Targeting IRFs for Immune Adjuvant Enhancement of Vaccine Immunogenicity

The overall goal of this contract is to use a high-throughput screening (HTS) program to identify and develop small molecule adjuvants that activate interferon-regulatory factors (IRFs) that enhance protective immunity for vaccines to NIAID Category A-C viruses.
Role: PI

(NEW)
1 R13 AI120422-01 (Nelson) 7/01/2015 – 6/30/2016 (b)(4)
NIH/NIAID $6,000
International Herpesvirus Workshop

The project supports travel and registration fees for postdoctoral fellows and graduate students to attend the annual International Herpesvirus Workshops (IHW).
Role: PI

(b)(4) (Picker) 7/28/2014 – 8/31/2019 (b)(4)
$6,144,126
Collaboration for AIDS Vaccine Discovery (CAVD)
Development of Attenuated CMV Vectors for an HIV/AIDS Vaccine

The overall goal of this project is the development of an HCMV vector-based HIV/AIDS vaccine (comprised of one or more HIV insert-expressing HCMV vectors) that is optimized for safety, efficacy and manufacturability.
Role: Project Leader; Outcomes 2.2 and 2.3

(THIS AWARD)
5 U19 AI109680-02 (Whitley) 3/01/2014 – 2/28/2019 (b)(4)
NIH/NIAID $274,539
Antiviral Drug Discovery and Development Center: Project 1: Identification and Development of Anti-Flavivirus Lead Drug Candidates

This project is designed to identify and develop small molecule anti-viral therapeutics against two medically important flaviviruses--dengue virus and West Nile virus. Furthermore, we will emphasize the development of drugs that show activity against multiple flaviviruses, and possibly other virus families as well.
Role: Site PI; Project 1

BAA-NIAID-DAIT-HIHAI2010085 (Nikolich-Zugich) 4/01/2011 – 5/15/2016 (b)(4)
Subcontract Y562709 on NIH Contract No. HHSN272201100017C
NIH/NIAID $195,051
Protective Immunity in Special Populations: Interface between Innate and Adaptive Immunity

This contract is a renewal of ongoing studies of our group with Dr Nikolich-Zugich to characterize defects in the aged immune system.
Role: Subcontract PI

5 P01 AI094417-05 (Picker) 7/15/2011 – 6/30/2016 (b)(4)
NIH/NIAID $206,000
Development of an Effector-Memory T Cell AIDS Vaccine (Project 2: Attenuation of CMV Vector Pathogenicity and Transmission by Altering Viral Tropism)

The goal of this project is to determine whether genetically modifying CMV to limit its ability to replicate in cell types associated with disease and transmission, while retaining its ability to persist in cells important for eliciting immunity, will lead to a safe and effective vector for an HIV/AIDS vaccine. In this Program, we will modify CMV vectors and/or use complementary heterologous vaccines with CMV vectors to both increase the potency of CMV/SIV vectors so as to achieve rates of protection closer to 100% of vaccines, and reduce the pathogenicity and shedding potential of CMV vectors (while retaining immunogenicity), so as to achieve an effective vaccine that is safe enough for use in a general human population.
Role: Project 2 PI

(NEW)
1 R01 CA179921-01A1 (Moses) 5/01/2015 – 4/30/2020 (b)(4)
NIH/NCI $228,750
Heme Oxygenase-1 as a Tumor Factor and Therapeutic Target for Kaposi Sarcoma

The major goals of this project are to characterize the role of the host enzyme heme oxygenase-1 (HO-1) in KSHV pathogenesis and Kaposi sarcoma (KS), and to determine if HO-1 is a valid therapeutic target for KS.
Role: Co-Investigator

INACTIVE
1 R56 AI105062-01 (Goodrum) 9/01/2013 – 7/31/2015 (b)(4)
NIH/NIAID (no-cost extension)
Antagonistic Viral Determinants Regulating the Outcome of Infection

The goal of this work is to determine the role of virus-host interactions identified by Dr. Goodrum in viral persistence. Dr. Goodrum will provide recombinant viruses. We will perform the experiments in NSG mice we create and harvest tissues for analysis.
Role: Subcontract PI

(b)(4) (Picker) 9/15/2011 – 6/30/2015 (b)(4)
(no-cost extension)
Collaboration for AIDS Vaccine Discovery (CAVD)
Development of an Attenuated CMV Vector for an HIV/AIDS Vaccine

The goal of this project is to construct HCMV/HIV vector homologues of the interim and optimized designs in Objective 2 and to determine whether these homologues exhibit analogous replication, tropism characteristics and level of insert expression compared to the *in vivo*-validated RhCMV/SIV version. Assessment of the interim vector designs will provide go/no-go criteria for further development, as only designs that work in the context of HCMV will move forward. Assessment of the final optimized version will provide crucial information for regulatory approval and clinical translation. The ultimate aim is to develop a safe and effective HIV/AIDS vaccine for use in high burden countries.
Role: Project Leader, Objective 3

1 R13 AI113945-01 (Nelson) 7/01/2014 – 6/30/2015 (b)(4)
NIH/NIAID $8,000
International Herpesvirus Workshop

The project supports travel and registration fees for postdoctoral fellows and graduate students to attend the annual International Herpesvirus Workshops (IHW).
Role: PI

5 U54 AI081680-05 (Nelson) 4/20/2009 – 2/28/2015 (b)(4)
NIH/NIAID (no-cost extension)
Pacific Northwest Regional Center of Excellence in Biodefense and Emerging Infectious Diseases

The goal of the PNWRCE is to identify age-related immune system defects to develop new vaccines and supplemental therapies to enhance protection of individuals to NIAID Category A-C pathogens. A second goal

of this center is to use systems genetic, chemical, and proteomics approaches to identify therapeutic targets for biodefense and emerging diseases.
Role: PI

1 R13 AI108033-01 (Nelson) 7/15/2013 – 6/30/2014 (b)(4)
NIH/NIAID $4,000
International Herpesvirus Workshop
The project supports travel and registration fees for postdoctoral fellows and graduate students to attend the 38th International Herpesvirus Workshop (IHW) in Grand Rapids, Michigan, United States, in 2013.
Role: PI

5 R01 HL088603-05 (Nelson) 5/01/2008 – 1/31/2013 (b)(4)
NIH/NHLBI $413,165
The Role of the Cytomegalovirus Secretome in the Acceleration of Transplant Vascular Sclerosis
In this project we propose the use of viral genetics to unravel the wound healing and angiogenesis mechanisms involved in CMV acceleration of TVS and CR.
Role: PI

OVERLAP No overlap

Other Research Support **Mark Prichard, UAB**

ACTIVE

HHSN272201100016I (Prichard) 06/01/2011 – 05/31/2018
NIH/NIAID (Base Contract)

The goals of this contract are to evaluate compounds against human DNA viruses

Task Order B22 HHSN27200009 (Prichard) 9/30/15– 9/29/16 (b)(4)
NIH/NIAID $1,063,630

HHSN2722010000271 (Quenelle) 9/30/134– 9/29/15 (b)(4)
NIH/NIAID $614,144
Animal models for herpes simplex virus and human cytomegalovirus

HHSN272201100034C (Whitley 1) 9/28/11-9/27/16 (b)(4)
NIH/NIAID $3,385,690
Adaptive sequential study evaluating prevention of neonatal HSV: Detection of maternal shedding.

HHSN272201100035C (Whitley 2) 9/28/11-9/27/16 (b)(4)
NIH/NIAID $2,065,894
A Phase II 6 Weeks Oral Valganciclovir versus Placebo in Infants with Congenital CMV Infection.

HHSN272201100036C (Whitley 3) 9/28/11-9/27/15 (b)(4)
NIH/NIAID $2,188,470
Safety Tolerability and Pharmacokinetics of CMX001 in Renal Transplant Recipients with BKV.

HHSN272201100037C (Whitley 4) 9/28/11-9/27/16 (b)(4)
NIH/NIAID $1,779,753
A Pharmacokinetic/Pharmacodynamic and Resistance Evaluation of Intravenous Ganciclovir in Infants.

HHSN272201100038C (Whitley 5) 9/28/11-9/27/16 (b)(4)
NIH/NIAID $1,967,277
Adaptive study of CMX001 in infants with neonatal herpes simplex virus (HSV)

2R44AI100401-03 SBIR Phase 2 (Amidon, TSRL : 12/1/14-11/30/16 (b)(4)
NIH/NIAID $87,172
The goal of the research is to evaluate broad spectrum antiviral drugs against the DNA viruses

1U19AI 109680-01 (Whitley 6) 1/1/14-12/31/18 (b)(4)
NIH/NIAID $34,138,453
The goal of the research is to support influenza studies for CETR Grant

(b)(4) 10/14/14-10/13/15 (b)(4)
$91,610
The goal of the research is In Vitro Antiviral Testing

(b)(4)

11/8/13-2/7/16

Combined Efficacy of CMX001 and Acyclovir against C\ **$15,708**

OVERLAP

NONE

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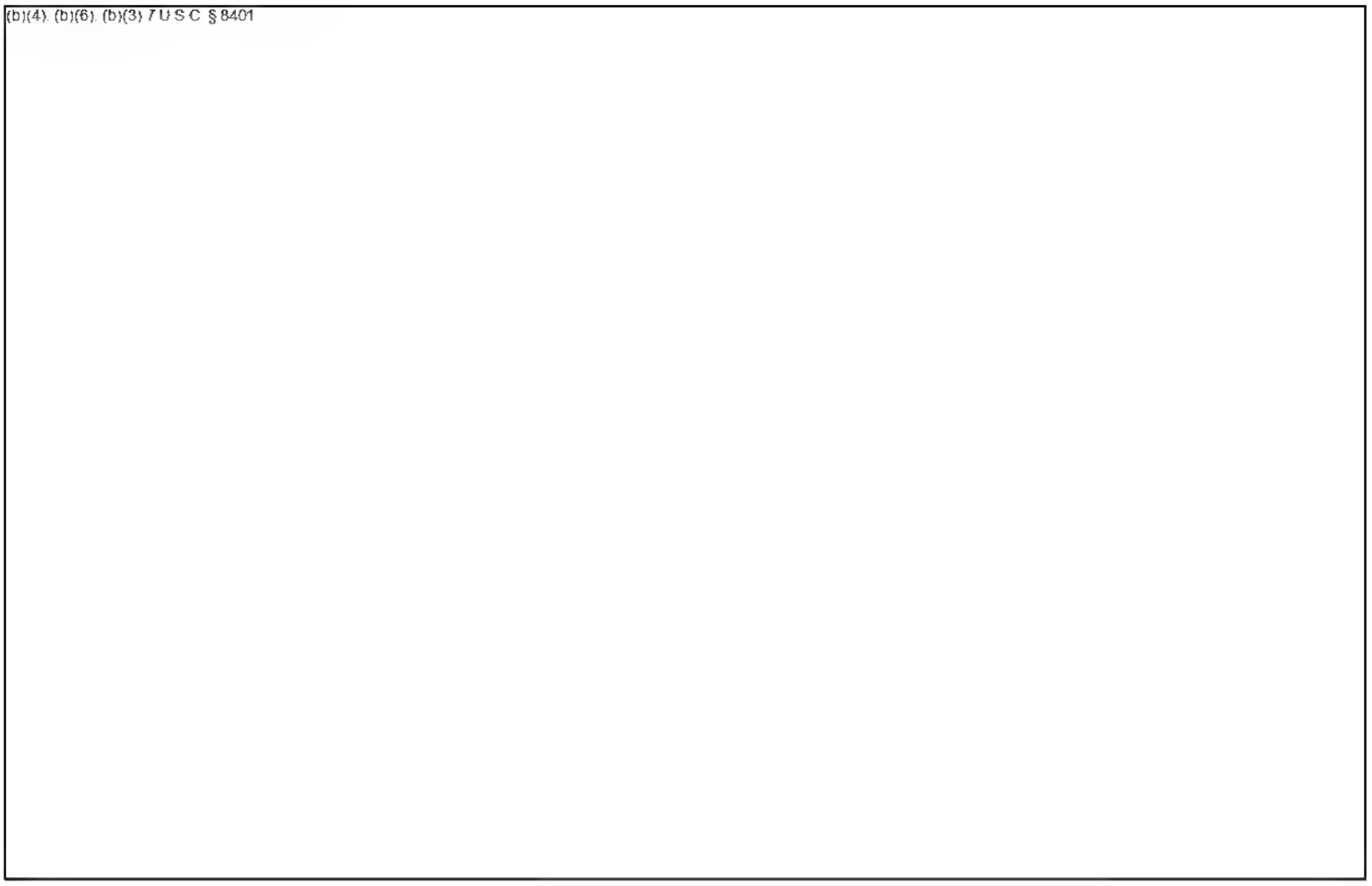
(b)(4) (b)(6) (b)(3) 7 U.S.C § 8401

of the Freedom of Information and Privacy Act

(b)(6) (b)(3) 7 U S C § 8401



OTHER SUPPORT FOR ALL KEY PERSONNEL – OREGON HEALTH & SCIENCE UNIVERSITY

STREBLOW, D

ACTIVE

(NEW)
R56 AI116633-01 (Streblow) 9/02/2015 – 8/31/2016
NIH/NIAID $250,000
(b)(4)
Characterizing the Role of CMV Latency in Solid Organ Transplant Rejection
The main goal of this project is to determine the mechanisms of cytomegalovirus-mediated solid organ transplant rejection.
Role: PI

(THIS AWARD; Project 3B)
5 U19 AI109680-02 (Whitley) 3/01/2014 – 2/28/2019
NIH/NIAID $299,955
(b)(4)
Antiviral Drug Discovery and Development Center
Project 3B: Novel Therapeutic Strategies Targeting Re-emerging Alphaviruses
The main goal of this project is to develop novel nucleoside and nucleotide inhibitors directed against Alphaviruses including Chikungunya virus and Venezuelan Equine Encephalitis virus.
Role: Project Leader; Project 3B

(b)(4) (Picker) 9/22/2014 – 9/30/2017
$1,578,446
(b)(4)
MHC II- and MHC E-restricted CD8+ T Cells and Control of HIV
The goal of this project is to provide fundamental research on a new type of vaccine-elicited CD8+ T cell immunity with the potential to control and clear HIV, and therefore to enable development of a safe and effective HIV/AIDS vaccine for use in high burden countries.
Role: Project Manager; Outcome 8 Activity 4.3

(b)(4) (Picker) 7/28/2014 – 8/31/2019
$6,144,126
(b)(4)
Development of Attenuated CMV Vectors for an HIV/AIDS Vaccine
The overall goal of this project is the development of an HCMV vector-based HIV/AIDS vaccine (composed of one or more HIV insert-expressing HCMV vectors) that is optimized for safety, efficacy and manufacturability.
Role: Project Manager; Outcome 5 Activity 1.5

(NEW)
5 P50 CA097186-12 6/01/2015 – 5/31/2016
Fred Hutchinson Cancer Research Center/Prostate Cancer Research Institute/
Knight Cancer Institute-NIH/NCI $50,000
(b)(4)
PNW Prostate Cancer SPORE Developmental Research Program (DRP), Pilot Project Award
CMV-vectored Prostate Cancer Vaccine
We have generated a novel vaccine platform using cytomegalovirus as a vector that induces astonishing levels and breadth of effector memory T cell responses that we will use to attempt to break tolerance to self antigens associated with prostate cancer, in order to generate a curative prostate cancer immunotherapy.
Role: PI

1 R41 AI109927-01A1 (Streblow/Bruening) 4/01/2014 – 3/31/2016
TomegaVax, Inc.-NIH/NIAID Advanced Technology STTR (No-cost extension)
(b)(4)
CMV Vectored Herpes Simplex Vaccine
TomegaVax (PI Bruening) will generate wild type and spread-deficient murine CMV vaccine vectors that express fragments of HSV-2 ICP0 and ICP4. The Streblow Lab will test the immunogenicity and protective efficacy of CMV vaccine vectors directed against HSV-2 in mice.
Role: Co-PI

Departmental Support 7.20 calendar
Vaccine and Gene Therapy Institute

INACTIVE

(b)(4) (Picker) 9/15/2011 – 6/30/2015 0.0 calendar
(no cost extension)
Collaboration for AIDS Vaccine Discovery (CAVD)
Development of an Attenuated CMV Vector for an HIV/AIDS Vaccine

The goal of this project is to construct HCMV/HIV vector homologues of the interim and optimized designs in Objective 2 and to determine whether these homologues exhibit analogous replication, tropism characteristics and level of insert expression compared to the *in vivo*-validated RhCMV/SIV version. Assessment of the interim vector designs will provide go/no-go criteria for further development, as only designs that work in the context of HCMV will move forward. Assessment of the final optimized version will provide crucial information for regulatory approval and clinical translation. The ultimate aim is to develop a safe and effective HIV/AIDS vaccine for use in high burden countries.
Role: Assistant Scientist

Defined Research Agreement (Früh/Streblow) 7/01/2014 – 6/30/2015 (b)(4)
OHSU Knight Cancer Institute $50,000
CMV-Vectored Cancer Vaccines

The goal of this project is to use CMV vectors to break tolerance to the prostate cancer associated protein PAP and by doing so elicit protective immune responses to the development of prostate cancer and metastatic disease, which would save tens of thousands of lives per year in the U.S. alone and would limit the need for invasive surgery or chemotherapies that have undesired side-effects and consequences.
Role: Co-PI

R01 AI089591 (Diamond/Streblow) 9/01/2014 – 5/31/2015 (b)(4)
NIH/NIAID (Administrative Supplement) $114,255
Antibody-Based Therapy of Chikungunya Virus

The major goal of this project is to generate an antibody therapy to control Chikungunya virus disease.
Role: Subcontract PI

1 R41 AI109927-01A1 (Streblow/Bruening) 4/01/2014 – 3/31/2015 (b)(4)
NIH/NIAID $95,212
CMV Vectored Herpes Simplex Vaccine

TomegaVax (PI Bruening) will generate wild type and spread-deficient murine CMV vaccine vectors that express fragments of HSV-2 ICP0 and ICP4. The Streblow Lab will test the immunogenicity and protective efficacy of CMV vaccine vectors directed against HSV-2 in mice.
Role: Co-PI

5 U54 AI081680-05 (Nelson) 4/20/2009 – 2/28/2015 0.0 calendar
NIH/NIAID (no cost extension)
Pacific Northwest Regional Center of Excellence Developmental Research Project:
Identification of Age-Related Defects to CHIKV Infections in a NHP Model

This project seeks to uncover the immunological and virological basis underlying increased Chikungunya virus (CHIKV) disease severity in the elderly. CHIKV is a re-emerging alphavirus that is listed as a NIAID Group III-Category C. CHIKV infection results in debilitating arthralgia in infected individuals. The recent re-emergence of CHIKV has been associated with the acquired ability of the virus to replicate in a more widely spread mosquito vector as well as increased mortality in the elderly and newborn populations. Although several countries are at risk, including the United States, no approved therapeutics or vaccines for CHIKV exist yet.
Role: Developmental Research Project (DP007) PI

5 R01 HL085451-04 (Orloff) 12/01/2008 – 11/30/2013
NIH/NHLBI
Cytomegalovirus Chemokine Receptors in Transplant Vascular Sclerosis

The goal of this project is to determine the role of Cytomegalovirus (CMV)-encoded chemokine receptors in CMV-accelerated Transplant Vascular Sclerosis (TVS).
Role: Co-Investigator

OVERLAP No overlap

Research Support

Active

BAO# 28XS124, Suto (PI) 04/27/2009 - 6/26/2015 See Task Orders as listed below
Leidos (NCI)

Chemical Biology Consortium — Collaborative Drug Discovery Partnership with NCI. The goals of this NCI program are to provide a comprehensive research framework leading to the development of new anticancer drugs. The program focuses on the identification of new molecular targets, and the rapid development of these from medicinal chemistry through final drug development.

BAO # 28XS124, TO#8, Admin, Suto (PI) 01/25/2011 – 3/31/2016 (b)(4)
Leidos (NCI) $1,485,740 (total cost)

Admin support for the Chemical Biology Consortium activities that are not related to any other CBC Technical Task Order.

1U19AI109680, Whitley (PI) 03/01/2014 – 02/28/2019 (b)(4)
NIAID $524,335

Antiviral Drug Discovery and Development Center. The goals of this NIAID program are the development of antiviral drugs for the treatment of emerging and reemerging infections. Specifically, the focus will be on flaviviruses, alphaviruses, corona viruses and influenza. The goal is to identify compounds working through mechanisms that affect viral replication and develop these leads in a translational manner to new human therapeutics. Role: Co-PI Medicinal Chemistry Core

1R01CA175012-01A, Murphy-Ullrich (PI) 08/1/2014 – 07/31/2019 (b)(4)
UAB (NCI) $143,402

This proposal will combine mechanistic studies with drug discovery efforts to achieve our goal of identifying an orally active lead compound for treatment of Multiple Myeloma. We will further determine the role of the TSP1-TGF-β pathway in MM through use of immune competent and TSP1 null models, by comparison of lead compounds to global TGF-β inhibitors. Role: Co-investigator

(b)(4) Suto (PI) 07/14/2015- 7/14/2016 (b)(4)
$1,000,000

Targeting Hallmarks of Cancer for Oncology Drug Discovery. The goals of this State funded initiative are to develop and train newer generations of cancer researchers as well as to promote innovation and research within the state. The Drug Discovery Division at Southern Research is conducting cutting edge research on molecularly targeted cancer drug discovery.

1R21ES024705-01, Matalon (PI) 09/22/14 – 09/22/2016 (b)(4)
UAB (NIEHS) $14,125

Data show that mice over expressing the human form of the heme oxygenase (HO)-1 gene and protein (hHO-1 BAC) exhibit significantly lower mortality when returned to room air post Br2 exposure as compared to their wild-type littermate controls. We have identified compounds that are potent inducers of the human HO-1 gene. The goals of this application are to establish the role of HO-1 in protecting mice from Br2 induced injury and test the efficacy of these compounds.
Role: Co-investigator

(b)(4) Suto, PI
(b)(4)

05/01/2015 – 04/30/2016
$76,909

The goal is to develop a primary and backup series of small molecules that could be further developed for use in the treatment of ALS. We will identify a primary lead series that is novel, orally bioavailable, and has CNS penetration using a directed medicinal chemistry effort focused on a current lead compound. The best candidates will be evaluated in vivo in the SOD-1 G93A ALS animal model to assess efficacy and target engagement.

Suto (PI)
(b)(4)

9/14/2015 – 9/13/2020
$1,187,439

(b)(4)

Goal: The goal is to identify novel read-through drugs for the treatment of cystic fibrosis. Initially, a large high-throughput screen will be run and the compounds further evaluated in several mechanistic assays. Lead optimization and profiling of the compounds will be initiated to identify a preclinical candidate.

U54TR001368-01 (Kimberly)
NIH/NCATS
UAB Center for Clinical and Translational Science (CCTS)
Role: Co-Investigator

9/1/15-8/31/20
$6,324,075 (UL1, KL2, TL1)

(b)(4)

Goal: The UAB CCTS will enhance human health by driving scientific discovery and dialogue across the bench, bedside and community continuum. The CCTS support this overall mission in a highly integrative network of relationships. Success in creating such an environment is dependent upon success in achieving five strategic priorities: 1) enhancing research infrastructure; 2) promoting investigator education, training and development; 3) accelerating discovery across the T1 interface; 4) expanding value-added partnerships; and 5) building sustainability.

Pending

(b)(4)

E. OVERALL IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

F. OVERALL CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

File uploaded: Umbrella F3.pdf

F.3: Significant changes to select agents

Following CDC approval, the recombinant SARS CoV (Urbani strain) expressing a NanoLuc reporter was transferred to (b)(6), (b)(3) 7 U S C § 8401 This virus is currently being used in assays to evaluate newly synthesized compounds for development of SAR in Core B.

G. OVERALL SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

File(s) uploaded
G1 Core B [redacted].pdf
G1 Project 3 Streblow.pdf
G1 Core C Pathak.pdf
G1 Project 2 [redacted].pdf
G1 Core A Whitley.pdf
G1 Project 4 Whitley.pdf
G1 Umbrella Whitley.pdf
G1 Project 1 Nelson.pdf

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Are there personnel on this project who are newly involved in the design or conduct of human subjects research?

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Does this project involve vertebrate animals?

Yes

G.8 PROJECT/PERFORMANCE SITES

Organization Name:	DUNS	Congressional District	Address
Primary: UNIVERSITY OF ALABAMA AT BIRMINGHAM	063690705		UNIVERSITY OF ALABAMA AT BIRMINGHAM 1720 2nd Ave. South BIRMINGHAM AL 35294

Oregon Health & Science University	096997515	OR-003	3181 SW Sam Jackson Park Rd. Portland OR 97239
Vanderbilt University	004413456	TN-005	3319 West End Avenue Suite 100 Nashville TN 37203
THe University of North Carolina at Chapel Hill	608195277	NC-004	Administrative Office Bldg Suiste 2200 104 Airport Rd, CB 1350 Chapel Hill NC 27599
University of Colorado at Denver	041096314	CO-006	1300 E. 17th Place, Room W1126 Anschutz Medical Campus Bldg 500 Denver CO 80045
Portland VA Research Foundation	827052887	OR-01	3710 SW US Veterans Hospital Rd Portland OR 97239
Southern Research Institute	006900526	AL-006	2000 Ninth Avenue South Birmingham AL 35205
Washington University	068552207	MO-001	Campus Box 8051 660 S. Euclid St. Louis MO 63110
UNIVERSITY OF ALABAMA AT BIRMINGHAM	063690705		UNIVERSITY OF ALABAMA AT BIRMINGHAM 1720 2nd Ave South BIRMINGHAM AL 352331806
UNIVERSITY OF ALABAMA AT BIRMINGHAM	063690705		UNIVERSITY OF ALABAMA AT BIRMINGHAM 1720 2nd Ave. South BIRMINGHAM AL 35294
Oregon Health & Science University	096997515	OR-003	3181 SW Sam Jackson Park Rd. Portland OR 97239
Vanderbilt University	004413456	TN-005	3319 West End Avenue Suite 100 Nashville TN 37203
THe University of North Carolina at Chapel Hill	608195277	NC-004	Administrative Office Bldg.Suiste 2200 104 Airport Rd, CB 1350 Chapel Hill NC 27599
University of Colorado at Denver	041096314	CO-006	1300 E. 17th Place, Room W1126 Anschutz Medical Campus Bldg 500 Denver CO 80045
Southern Research Institute	006900526	AL-006	2000 Ninth Avenue South Birmingham AL 35205
Washington University	068552207	MO-001	Campus Box 8051 660 S. Euclid St. Louis MO 63110

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

G.10.a Is it anticipated that an estimated unobligated balance (including prior year carryover) will be greater than 25% of the current year's total approved budget?

No

G.11 PROGRAM INCOME

Is program income anticipated during the next budget period? No
G.12 F&A COSTS Not Applicable

Core specific information

Project 1 (flavivirus)

Dengue Virus: Following completion of the HTS campaign for DENV, a list of 45 confirmed and validated hits was provided to project team 1 investigators and the MCLDC team for further investigation and design of new compounds. The Screening Core B has developed and implemented a secondary assay using laser scanning microscopy to measure viral protein expression in HEK293 cells to support evaluation of newly synthesized compounds and development of SAR.

West Nile Virus: In agreement with project team 1 investigators, we have decided to use HEK cells expressing IFIT induced by doxycycline for the HTS campaign (cell line produced in the Diamond lab).

Project 2 (coronavirus)

SARS: Following completion of the HTS campaign for SARS, a list of 874 confirmed and validated hits was provided to project team 1 investigators and the MCLDC for further investigation and design of new compounds. The Screening Core, in consultation with the (b)(6) (b)(3) 7 ab, implement a secondary assay using the SARS nanoluc virus to support evaluation of newly synthesized compounds and development of SAR.

Project 3 (alphavirus)

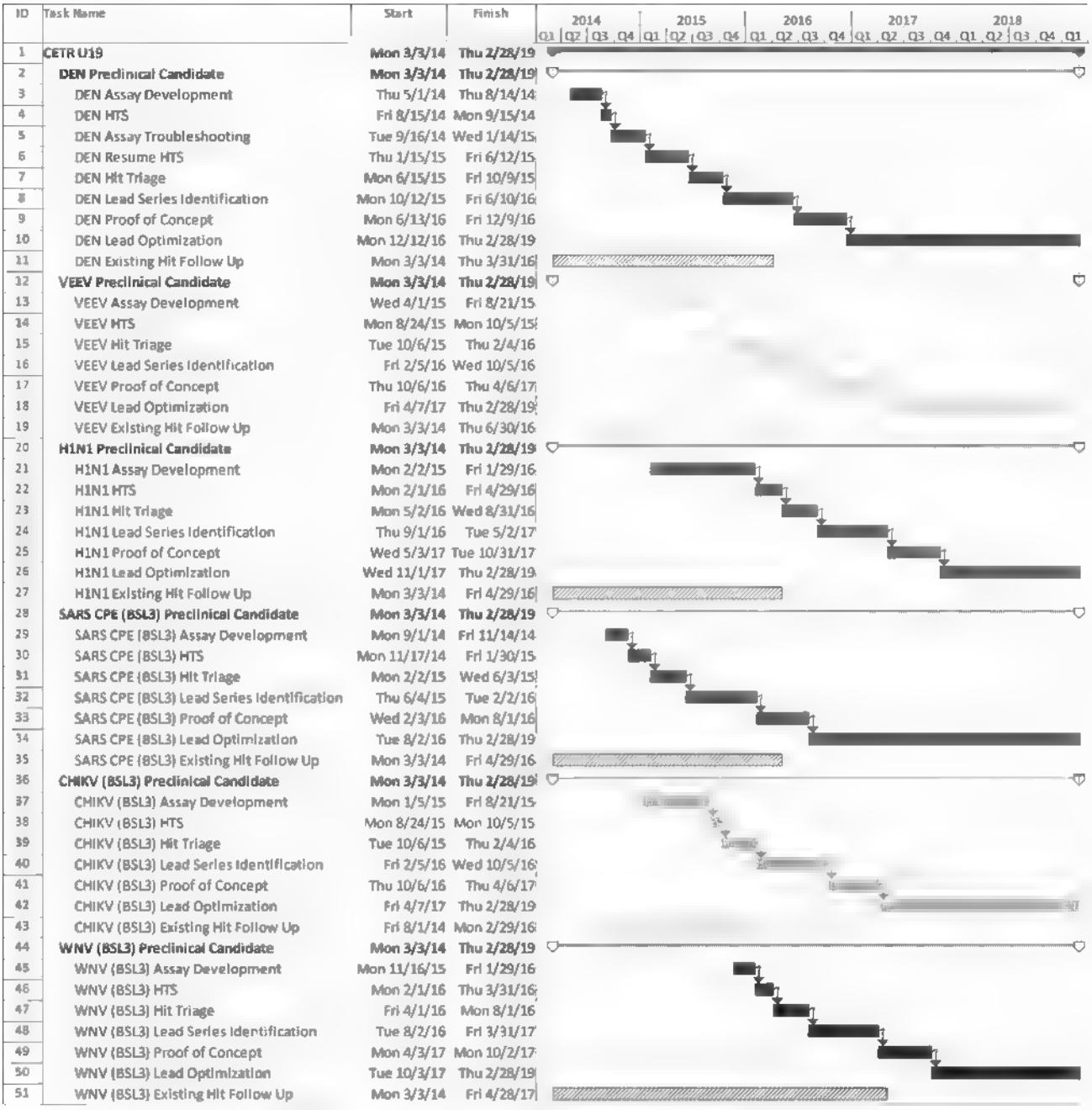
Chikungunya virus: Following discussion and agreement with the project PIs, the Screening Core will develop and implement an SAR driving assay employing a vaccine CHIKV virus strain (BSL-2) producing mKATE, a fluorescent protein reporter (virus obtained from the Heise lab).

Venezuela Equine Encephalitis virus: The CPE assay employed in HTS will also be used to drive SAR in this project.

Project 4 (influenza virus)

H1N1: The NanoLuc reporter virus is constructed with A/California/7/2009 (H1N1). The hits identified using this virus will be counter screened for anti-viral activity against H3N2 using the HEK293 luciferase reporter assay and against H5N1 (high path) virus using a CPE assay in MDCK cells.

Timeline of activities



ID	Task Name	Start	Finish
1	**CETR U19**	**Mon 3/3/14**	**Thu 2/28/19**
2	**DEN Preclinical Candidate**	**Mon 3/3/14**	**Thu 2/28/19**
3	DEN Assay Development	Thu 5/1/14	Thu 8/14/14
4	DEN HTS	Fri 8/15/14	Mon 9/15/14
5	DEN Assay Troubleshooting	Tue 9/16/14	Wed 1/14/15
6	DEN Resume HTS	Thu 1/15/15	Fri 6/12/15
7	DEN Hit Triage	Mon 6/15/15	Fri 10/9/15
8	DEN Lead Series Identification	Mon 10/12/15	Fri 6/10/16
9	DEN Proof of Concept	Mon 6/13/16	Fri 12/9/16
10	DEN Lead Optimization	Mon 12/12/16	Thu 2/28/19
11	DEN Existing Hit Follow Up	Mon 3/3/14	Thu 3/31/16
12	**VEEV Preclinical Candidate**	**Mon 3/3/14**	**Thu 2/28/19**
13	VEEV Assay Development	Wed 4/1/15	Fri 8/21/15
14	VEEV HTS	Mon 8/24/15	Mon 10/5/15
15	VEEV Hit Triage	Tue 10/6/15	Thu 2/4/16
16	VEEV Lead Series Identification	Fri 2/5/16	Wed 10/5/16
17	VEEV Proof of Concept	Thu 10/6/16	Thu 4/6/17
18	VEEV Lead Optimization	Fri 4/7/17	Thu 2/28/19
19	VEEV Existing Hit Follow Up	Mon 3/3/14	Thu 6/30/16
20	**H1N1 Preclinical Candidate**	**Mon 3/3/14**	**Thu 2/28/19**
21	H1N1 Assay Development	Mon 2/2/15	Fri 1/29/16
22	H1N1 HTS	Mon 2/1/16	Fri 4/29/16
23	H1N1 Hit Triage	Mon 5/2/16	Wed 8/31/16
24	H1N1 Lead Series Identification	Thu 9/1/16	Tue 5/2/17
25	H1N1 Proof of Concept	Wed 5/3/17	Tue 10/31/17
26	H1N1 Lead Optimization	Wed 11/1/17	Thu 2/28/19
27	H1N1 Existing Hit Follow Up	Mon 3/3/14	Fri 4/29/16
28	**SARS CPE (BSL3) Preclinical Candidate**	**Mon 3/3/14**	**Thu 2/28/19**
29	SARS CPE (BSL3) Assay Development	Mon 9/1/14	Fri 11/14/14
30	SARS CPE (BSL3) HTS	Mon 11/17/14	Fri 1/30/15
31	SARS CPE (BSL3) Hit Triage	Mon 2/2/15	Wed 6/3/15
32	SARS CPE (BSL3) Lead Series Identification	Thu 6/4/15	Tue 2/2/16
33	SARS CPE (BSL3) Proof of Concept	Wed 2/3/16	Mon 8/1/16
34	SARS CPE (BSL3) Lead Optimization	Tue 8/2/16	Thu 2/28/19
35	SARS CPE (BSL3) Existing Hit Follow Up	Mon 3/3/14	Fri 4/29/16
36	**CHIKV (BSL3) Preclinical Candidate**	**Mon 3/3/14**	**Thu 2/28/19**
37	CHIKV (BSL3) Assay Development	Mon 1/5/15	Fri 8/21/15
38	CHIKV (BSL3) HTS	Mon 8/24/15	Mon 10/5/15
39	CHIKV (BSL3) Hit Triage	Tue 10/6/15	Thu 2/4/16
40	CHIKV (BSL3) Lead Series Identification	Fri 2/5/16	Wed 10/5/16
41	CHIKV (BSL3) Proof of Concept	Thu 10/6/16	Thu 4/6/17
42	CHIKV (BSL3) Lead Optimization	Fri 4/7/17	Thu 2/28/19
43	CHIKV (BSL3) Existing Hit Follow Up	Fri 8/1/14	Mon 2/29/16
44	**WNV (BSL3) Preclinical Candidate**	**Mon 3/3/14**	**Thu 2/28/19**
45	WNV (BSL3) Assay Development	Mon 11/16/15	Fri 1/29/16
46	WNV (BSL3) HTS	Mon 2/1/16	Thu 3/31/16
47	WNV (BSL3) Hit Triage	Fri 4/1/16	Mon 8/1/16
48	WNV (BSL3) Lead Series Identification	Tue 8/2/16	Fri 3/31/17
49	WNV (BSL3) Proof of Concept	Mon 4/3/17	Mon 10/2/17
50	WNV (BSL3) Lead Optimization	Tue 10/3/17	Thu 2/28/19
51	WNV (BSL3) Existing Hit Follow Up	Mon 3/3/14	Fri 4/28/17

G1 Project 3 – Streblow

1. Significant Changes in Specific Aims

Not Applicable.

2. Significance of the Work

Our Program aims to develop novel antiviral agents to emerging human viral pathogens. This Project will develop broad-spectrum nucleoside/nucleotide inhibitors against Alphaviruses with a focus on Chikungunya virus and Venezuelan Equine Encephalitis virus, both of which are human pathogens that cause severe disease and are associated with mortality and with no currently FDA approved vaccine or therapeutics for treatment.

3. Product Development Milestones

Not Applicable.

4. Significant Project-Generated Resources

1. **THF-ΔIRF-3:** Human foreskin fibroblasts telomerized with pBABE lentivector from AddGene. These cells constitutively express the reverse Tet-transactivator via lentivector (Clontech # 631069); not relevant for this study but just FYI. The IRF3 gene sequence has been disrupted using the CRISPR/Cas9 system (AddGene vector # 49535). The CRISPR lentivector confers resistance to puromycin, which should always be maintained in the culture media @ 3ug/mL (Invivogen Cat # ant-pr-1). The cells are frozen down at 1.8 x 10^6 per vial and can be brought up directly into a T75 + 14mL media. Once confluent they can be split 1:10 for expansion or maintenance. Culture media is 1x DMEM (Fisher Cat#MT-10-017-CV) with 1x pen/strep and 10% FCS (we've used many vendors, e.g. Life Technologies). Cell line constructed by Dr. DeFilippis.
2. **THF-ΔIFIT1, THF-ΔIFIT2, THF-ΔSTING, THF-ΔIPS1, THF-ΔSTAT1:** Human foreskin fibroblasts telomerized with pBABE lentivector from AddGene. These are also stably transduced with a firefly luciferase-coding region under the control of the interferon responsive element using a lentivector obtained from System Biosciences. Individual cell lines were constructed in which the protein coding regions for IFIT1, IFIT2, STING, IPS1, or STAT1 were disrupted using the CRISPR/Cas9 system (AddGene vector # 52961). The CRISPR lentivector confers resistance to puromycin, which should always be maintained in the culture media @ 3ug/mL (Invivogen Cat # ant-pr-1). The cells are frozen down at 1.8 x 10^6 per vial and can be brought up directly into a T75 + 14mL media. Once confluent they can be split 1:10 for expansion or maintenance. Culture media is 1x DMEM (Fisher Cat#MT-10-017-CV) with 1x pen/strep and 10% FCS (we've used many vendors, e.g. Life Technologies). Cell lines constructed by Dr. DeFilippis.
3. **CHIKV Caribbean Strain Infectious Clone:** $CHIKV_{99659}$ was recently isolated from the British Virgin Islands in December of 2013. A low-passage stock of this strain was provided to the members of the Alphavirus group from Dr. Michael Diamond (Project 2). The Heise lab, in collaboration with Dr. Nathanial Moorman at UNC, has sequenced the isolate and constructed an infectious clone of the virus.
4. **$CHIKV_{181/25}$ Strains Expressing nano-Luciferase (nLuc):** Into the infectious clone of $CHIKV_{181/25}$ was introduced an in-frame nLuc reporter gene. Two different viruses were constructed by the Heise Lab: pTH1.2 (NSP-3nLuc) and pTH2.1 (Capsid-nLuc), which will be utilized by SR for cherry-pick validation screens and for mechanism of action studies.
5. **$CHIKV_{AF15561}$ strain expressing mKate:** An in-frame mKate reporter gene was cloned into the infectious clone of the pathogenic parental virus of $CHIKV_{181/25}$ ($CHIKV_{AF15561}$). Constructed by Dr. Morrison's group.
6. **G10:** A novel small molecule (4-(2-chloro-6-fluorobenzyl)-N-(furan-2-ylmethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazine-6-carboxamide) capable of blocking Alphavirus replication by activating STING-dependent activity in human cells was characterized and described by Dr. DeFilippis.

Core Specific Information.

Activities for Core C have included: 1) analysis of high throughput screening data; 2) purchase and re-synthesis of promising hits for potential follow-up chemistry; 3) distribution of these compounds to the various Research Projects for evaluation in relevant assays; 4) medicinal chemistry on selected and confirmed hits (hit-to-lead chemistry); and, 5) structure-based virtual screening of commercial libraries against relevant X-ray-derived models. Below is a summary of Core C interactions with the four individual Research Projects with respect to these activities.

1. Research Project 1 (Flaviviruses):

Dengue Virus (DENV): Continuing from Yr 1 on MLPCN (Molecular Libraries Program) hits, 19 compounds were acquired and tested against DENV in a confirmatory dose-response assay. Two compounds, SRI-34353 and SRI-33613, showed an EC_{90} of 0.77 μM and 0.03 μM with a CC_{50} (cytotoxicity) > 40 μM. These compounds also showed activity in a new mirror ball (MB) assay that is now being used for testing newly synthesized analogs of the hit compounds. The other 17 hits showed significant cellular toxicity and were eliminated. The high throughput data from the 300K+ AD3C screen was also analyzed and 55 compounds were selected based on potency and toxicity data. These 55 compounds were further triaged using PAINS (pan-assay inhibitors) filtering and removing any other promiscuous, cytotoxic, or undesirable compounds. These analyses left 11 compounds remaining as potential lead comounds. New samples of these 11 hit compound were acquired commercially or by re-synthesis and all were shipped recently to Research Project 1 lab for re-confirmation in the CPE and viral load reduction assays. Simultaneously, these 11 samples are also being reconfirmed in mirror-ball (MB) SAR assay at SR. ADME properties, such as aqueous solubility, log D, and mouse and human microsomal stability, will be evaluated on all confirmed hits before initiating chemistry on two of the best lead compounds. In another study, 93 commercially acquired compounds from a total of 7.5 million compounds that were screened in a structure-based virtual screen (SBVS) against the methyltransferase domain of the Dengue virus NS5 protein (PDB 3P8Z) were tested against DENV at 10 μM concentration and were found to be inactive.

West Nile Virus (WNV): The results from a 288,000-compound MLPCN single-point assay screen against West Nile virus was reviewed and followed by a dose-response assay resulted in 62 active compounds. As described above for DENV, after filtering out any undesirable and cytotoxic agents, 19 compounds remained which were then acquired commercially or re-synthesized and tested in Research Project 2 lab. Only two compounds, SRI-33625 and SRI-22003, showed significant antiviral activity: EC_{90} = 0.1 μM and EC_{90} = 3.0 μM, respectively with a CC_{50} > 25 μM. These compounds are currently being evaluated for *in vitro* ADME properties before starting hit-to-lead chemistry.

2. Research Project 2 (Coronaviruses)

Using the methodology described above for Project 1, 38 compounds were acquired or re-synthesized after evaluating the results of a previous 102,000-compound high throughput SARS virus screen. These 38 compounds were tested in a re-confirmation NanoLuc (NL) assay and a Luciferase-based assay at SR and in Research Project 2 lab. Six compounds showed antiviral activity with an EC_{50} < 10 μM and 3 of these 6 compounds showed ≥ 2 log reduction in viral load reduction (VLR) assay. Earlier this year we received HTS assay results from a 300K+ compound AD3C screen. The compounds from this screen were then run in a dose response assay, a PAINS filtering was applied and other promiscuous and undesirable compound were removed. From this filtering and dose response assay, a total of 157 compounds were selected that had an EC_{50} < 20 μM and a CC_{50} > 30 μM. New samples of 69 of these hits were acquired or re-synthesized and results from the NL assay (in duplicate) were analyzed. Sixteen compounds were then selected for testing in the viral load reduction assay that had an EC_{50} < 15 μM and a CC_{50} > 30 μM. Two compounds showed 2 or more log reduction

in viral load. Re-confirmation activity assays are currently being run on another 88 compounds which were acquired recently from commercial sources. In another study, 77 commercially available compounds from a 7.5 million compounds in a structure-based virtual screen (SBVS) against the SARS virus, 2'-*O*-methyltransferase protein (PDB 3R24), were tested at 10 μM concentration and were found to be inactive.

Based on the activity data from the NL and viral load reduction assays, 4 compounds, SRI-27298 (HTS-ADR EC_{50} = 4.7 μM, NL EC_{50} = 4.5 μM, VLR = 4.8), SRI-35020 (HTS-ADR EC_{50} = 0.55 μM, NL EC_{50} = 1.6 μM, VLR = 3.2), SRI-35293 (HTS-ADR EC_{50} = 1.0 μM, NL EC_{50} = 12.3 μM, VLR = 2.3) and SRI-35610 (HTS-ADR EC_{50} = 6.5 μM, NL EC_{50} = 14.3 μM, VLR = 2.1) were selected for hit-to-lead chemistry. ADME data on these 4 hit compounds indicates low solubility and poor mouse metabolic stability A target profile of a lead compound should have an EC_{50} < 100 nM (EC_{90} < 500 nM), a CC_{50} > 20 μM, microsome stability (mouse) with $t_{1/2}$ > 20 min and solubility ≥ 10 μM. A total of 192 analogs of these 4 hits have been synthesized to date of which none meet all of these targeted parameters. However, promising medicinal chemistry approaches continue with additional analogs being synthesized.

3. Research Project 3 (Alphaviruses)

Venezuelan Equine Encephalitis Virus (VEEV): In continuation from Yr 1, 35 hits were selected from the results of a 384,000-compound MLPCN screen and the filtering process as described above (in Research Project 1) was applied. These 35 compounds were then tested in Research Project 3 lab in a Normal Human Dermal Fibroblasts (NHDF) cell line against VEE virus. Three compounds were re-confirmed for activity that had an EC_{90} < 10 μM,but SRI-33394 showed excellent antiviral activity (EC_{90} = 0.7 μM and VLR = 6.5). However, this compound has a t ½ = 2.1 min in mouse microsomes. This microsomal instability is most likely attributed to the presence of a thiourea and furan functionality in the molecule. Thus, approximately 70 rationally designed analogs of SRI-33394 were synthesized. Based on the antiviral data of these analogs, the sulfur of the thio urea deemed very important for activity. The current lead compound, SRI-34339 (EC_{90} = 0.01 μM and VLR = 8.3), possesses a thiourea group and has a t ½ = 1.3 min in mouse microsomes. Hence, new approaches to synthesize analogs of SRI-34339 that replace or mimic the thiourea are underway. It is anticpated that these new analogs will have better microsomal mouse stability and improved antiviral activity.

Chikungunya Virus (CHIKV): Thirty five VEEV hits from MLPCN screen were also tested against CHIKV. Four compounds (out of these 35 hits) showed CHIKV activity. . The three most active compounds (SRI-33366, SRI-33377 and SRI-33392) were selected for follow-up. A total of 250 synthesized and commercially available analogs of these 3 actives were tested for antiviral activity using the NHDF (normal human dermal fibroblasts) cell line in Research Project 3 lab. Based on the VLR data of these compounds, chemistry was initiated on SRI-33366 (EC_{90} = 3.2 μM and VLR = 1.7). Rationally designed 80 analogs of SRI-33366 were synthesized and tested in the antiviral and viral load reduction assays. The current lead compound is SRI-34963 which possesses good antivral activity (EC_{90} = 0.45 μM, CC_{50} > 25 μM, VLR value of 3.9) and reasonable ADME properties (mouse microsomal stability: t ½ = 11 min; solubility is 20 μM) Medicinal chemistry continues with a goal of identifying a compound to test in animals that has improved ADME properties and antiviral activity.

4. Research Project 4 (Influenza A virus)

Data from a previous in-house screen against influenza A virus of a 25,500-compound library was reviewed. Forty compounds were selected to test in the dose-response assay from which 19 compounds were confirmed actives. These 19 compounds were then filtered through PAINS and any undesirable compounds were removed. The remaining compounds were then tested by Research Project 4 which resulted in one compound, SRI-33941, with confirmed antiviral activity against virus

strains H1N1 (EC_{50} = 28.1 μM), H5N1 (28.1 μM), and H3N2 (19.8 μM). Approximately 220 analogs of SRI-33941 found in our screening library and 10 very specific targeted compounds (that were not present in the screening set) were synthesized and all were tested. SRI-34518 was identified from this testing which showed enhanced activity against all three virus strains [H1N1 (EC_{50} = 13.9 μM), H5N1 (7.5 μM), and H3N2 (3.7 μM)]. Rationally designed fragment analogs of SRI-34518 were also synthesized or commercially acquired and tested. From these fragments, SRI-34993 was identified which showed improved antiviral activity in two of the strains: H1N1 (EC_{50} = 3.2 μM) and H3N2 (EC_{50} = 2.9 μM) strains. Medicinal chemistry is in progress to identify compounds with sub-micromolar potency against all three virus strains and improved ADME properties than SRI-34993.

G.1 (G1 Project 2 (b)(6), (b)(3) 7 pdf)

G1 Project 2 – (b)(6), (b)(3) 7 U.S.C. § 8401

1. Signifance Changes in Specific Aims

Not Applicable.

2. Significance of the Work

In this Project we will use extensive small molecule libraries and a sensitive high-throughput in vitro screening assay to identify inhibitors of SARS-CoV replication fidelity and RNA capping that will lead to profound in vivo attenuation, and potentially represent broadly-efficacious inhibitors of endemic and emerging CoVs.

3. Product Development Milestones

Not Applicable.

4. Significant Project-Generated Resources

Not Applicable.

The Admin Core has served all Projects equally by the following activities:

- Scheduled and hosted monthly conference calls in which all projects provided brief updates and received input from the other personnel on the call to help in troubleshooting, if needed.
- Mediated transfer of funds to the institutions pending the signing of the consortium agreement so that work could be underway and invoiced.
- Coordinated preparation and execution of year two annual subaward amendments for all sites.
- Processed payments to sites following submission of invoices.
- Planned and hosted the second annual AD3C meeting in which all personnel got to meet each other in person, thereby facilitating further interactions between projects and cores.
- Arranged for Project Leaders of 3 of the 4 projects to attend the reverse site visit to NIH and present their project's progress; represented the progress of the remaining Project during the presentation.
- Communicated updates related to NIH grant issues, particularly those that impact the areas being investigated by AD3C investigators, e.g. select agent policies.
- Provided feedback from the External Advisory Board reviews to the Projects and Cores.

G1 Project 4 – Whitley

1. Significant Changes in Specific Aims

Not Applicable.

2. Significance of the Work

Influenza A viruses continue to emerge from the aquatic avian reservoir and cause seasonal epidemics and infrequent pandemics. Recent experimental evidence by our group and others support the development of novel antivirals targeting the influenza polymerase function. The discovery of molecules that inhibit influenza virus RNA replication is essential to complement the existing drug arsenal, which is proving less effective due to the increasing incidence of mutational resistance.

3. Product Development Milestones

Not Applicable.

4. Significant Project-Generated Resources

Not Applicable.

G1 Umbrella – Whitley

1. Product Development

Not Applicable. Even though our CETR included Product Development Strategy sections, the Projects are all very early in the process of drug discovery, and this Product Development section is therefore not applicable at this time.

2. Biocontainment/Security

Project 1

1. Project title: Identification and characterization of anti-flaviviral compounds
2. Project Leader: Jay Nelson
3. Collaborators: Michael Diamond, Alec Hirsch, Jessica Smith
4. BSL Laboratory employed: BSL3 (at both OHSU and Washington University)
5. Pathogens Evaluated: West Nile Virus, Dengue virus

Project 2

1. Project Title: Inhibitors of coronavirus fidelity and cap methylation as broadly applicable therapeutics
2. Project Leader: (b),(6), (b)(3),7 U.S.C. § 8401 (Vanderbilt)
3. Collaborator(s): Baric, RS (UNC)
4. BSL Laboratory Employed: BSL3 (Vanderbilt) & BSL3/ABSL3 (UNC)
5. Pathogen(s) Evaluated: Both Vanderbilt and UNC: SARS-CoV, MERS-CoV. Both Vanderbilt and UNC Select Agent Certified.

Project 3

1. Project title: Novel Therapeutic Strategies Targeting Re-emerging Alphaviruses
2. Project Leader: Daniel N. Streblow (OHSU)
3. Collaborators: (b)(6) (b)(3) 7 U S C § 8401 (UNC), Thomas Morrison (U Colorado Denver), Victor Defilippis (OHSU)
4. **Labs at the following sites were used:**
    1. **Vaccine & Gene Therapy Institute/Oregon Health & Science University.**
        a. BSL-3/VGTI rm2215A, Small Animal ABSL-3/VGTI, Nonhuman Primate ABSL-3 Building/ONPRC
        b. Pathogen: Chikungunya Virus
    2. **University of North Carolina-Chapel Hill.**
        a. (b)(3).7 U.S.C § 8401
        b. Pathogen: Chikungunya virus, Venezuelan equine encephalitis virus
    3. **University of Colorado-Denver.**
        a. BSL-3/UCD Anschutz Medical Campus, Small Animal ABSL-3/UCD Anschutz Medical Campus
        b. Pathogen: Chikungunya Virus

Core B utilized the BSL3 facility at Southern Research Institute for the following Projects:

Project number: 1
Project Title: Identification and Development of Anti-Flavivirus Lead Drug Candidates
Project Leaders: Michael Diamond
Collaborators:
BSL Laboratory Employed: Southern Research Institute (for HTS activities)
Pathogens Evaluated: West Nile (NY99)

Project number: 2
Project Title: Inhibitors of Coronavirus Fidelity and Cap Methylation as Broadly Applicable Therapeutics
Project Leaders: (b)(6), (b)(3) 7 U S C § 8401 Ralph Baric
Collaborators:
BSL Laboratory Employed: (b)(6), (b)(3) 7 U S C § 8401 (for HTS activities)
Pathogens Evaluated: SARS Toronto-2 and SARS Urbani/Nanoluc Clone

Project number: 3
Project Title: Novel Therapeutic Strategies Targeting Re-emerging Alphaviruses
Project Leaders: Daniel Streblow, (b)(6), (b)(3) 7 U S C § 8401
Collaborators:
BSL Laboratory Employed: Southern Research Institute (for HTS activities)
Pathogen Evaluated: CHIKV Sri Lanka strain

3. Follow-on Funding

Not Applicable

G1 Project 1 – Nelson

1. Signifance Changes in Specific Aims

Not Applicable.

2. Significance of the Work

The flaviviruses are associated with significant morbidity, mortality, and economic burden throughout world. Nevertheless, no specific anti-viral therapies for disease associated with these viruses are currently available. This project is designed to identify and develop small molecule anti-viral therapeutics against two medically important flaviviruses--dengue virus and West Nile virus. Furthermore, we will emphasize the development of drugs that show activity against multiple flaviviruses, and possibly other virus families as well.

3. Product Development Milestones

Not Applicable.

4. Significant Project-Generated Resources

Not Applicable.

Composite Application Budget Summary

Categories	Budget Period
Salary, Wages and Fringe Benefits	177,654
Equipment	0
Travel	51,808
Participant/Trainee Support Costs	0
Other Direct Costs (excluding Consortium)	55,152
Consortium Costs	6,732,432
Direct Costs	7,017,046
Indirect Costs	133,768
Total Direct and Indirect Costs	7,150,814

*This application includes at least one component led by an organization that has a DUNS different than the Applicant Organization. The indirect cost calculation for the applicant organization may not include all allowed Indirect Costs for the first $25K of requested consortium costs and, therefore, may appear less than expected. No action is required from the applicant; NIH will make any appropriate corrections to the budget calculations administratively. The application review will not be affected.

Component Budget Summary

Components	Categories	Budget Period
5318-001 (Admin Core)	Salary, Wages and Fringe Benefits	87,693
	Equipment	0
	Travel	48,808
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	32,155
	Consortium Costs	0
	Direct Costs	168,656
	Indirect Costs	79,268
TOTALS	Total Direct and Indirect Costs	247,924
5323-001 (Core)	Salary, Wages and Fringe Benefits	199,246
	Equipment	0
	Travel	2,500
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	352,698
	Consortium Costs	0
	Direct Costs	554,444
	Indirect Costs	407,765
TOTALS	Total Direct and Indirect Costs	962,209
5324-002 (Core)	Salary, Wages and Fringe Benefits	720,394
	Equipment	0
	Travel	5,000

	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	321,271
	Consortium Costs	0
	Direct Costs	1,046,665
	Indirect Costs	1,281,750
TOTALS	Total Direct and Indirect Costs	2,328,415
5325-001 (Project)	Salary, Wages and Fringe Benefits	80,624
	Equipment	0
	Travel	3,000
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	88,708
	Consortium Costs	0
	Direct Costs	172,332
	Indirect Costs	90 474
TOTALS	Total Direct and Indirect Costs	262,806
5327-002 (Project)	Salary, Wages and Fringe Benefits	134,186
	Equipment	0
	Travel	6,000
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	164,185
	Consortium Costs	0
	Direct Costs	304,371
	Indirect Costs	158,273
TOTALS	Total Direct and Indirect Costs	462,644

5328-003 (Project)	Salary, Wages and Fringe Benefits	90,092
	Equipment	0
	Travel	4,000
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	55,908
	Consortium Costs	0
	Direct Costs	150,000
	Indirect Costs	78 000
TOTALS	Total Direct and Indirect Costs	228,000
5321-004 (Project)	Salary, Wages and Fringe Benefits	128 448
	Equipment	0
	Travel	3,000
	Participant/Trainee Support Costs	10,000
	Other Direct Costs (excluding Consortium)	158 507
	Consortium Costs	0
	Direct Costs	299,955
	Indirect Costs	217,466
TOTALS	Total Direct and Indirect Costs	517,421
5322-005 (Project)	Salary, Wages and Fringe Benefits	89 961
	Equipment	0
	Travel	3,000
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	22,997
	Consortium Costs	0

	Direct Costs	115,958
	Indirect Costs	54,500
TOTALS	Total Direct and Indirect Costs	170,458
5319-006 (Project)	Salary, Wages and Fringe Benefits	207,939
	Equipment	0
	Travel	6,500
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	122,600
	Consortium Costs	0
	Direct Costs	337,039
	Indirect Costs	222,154
TOTALS	Total Direct and Indirect Costs	559,193
5320-007 (Project)	Salary, Wages and Fringe Benefits	212,835
	Equipment	0
	Travel	3,000
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	41,888
	Consortium Costs	0
	Direct Costs	257,723
	Indirect Costs	146,902
TOTALS	Total Direct and Indirect Costs	404,625
5329-008 (Project)	Salary, Wages and Fringe Benefits	74,596
	Equipment	0
	Travel	0

	Participant/Trainee Support Costs	6,000
	Other Direct Costs (excluding Consortium)	69,495
	Consortium Costs	0
	Direct Costs	150,091
	Indirect Costs	79,971
TOTALS	Total Direct and Indirect Costs	230,062
5330-009 (Project)	Salary, Wages and Fringe Benefits	229,776
	Equipment	0
	Travel	10,000
	Participant/Trainee Support Costs	0
	Other Direct Costs (excluding Consortium)	122,701
	Consortium Costs	0
	Direct Costs	362 477
	Indirect Costs	414 580
TOTALS	Total Direct and Indirect Costs	777 057
TOTALS		**7,150,814**

Categories Budget Summary

Categories	Components	Budget Period
R&R Budget - Senior/Key Person Funds Requested	5318-001 (Admin Core)	62,747
	5323-001 (Core)	42,132
	5324-002 (Core)	144,718
	5325-001 (Project)	11,080
	5327-002 (Project)	105,179
	5328-003 (Project)	19,670
	5321-004 (Project)	31,572
	5322-005 (Project)	57,850
	5319-006 (Project)	130,265
	5320-007 (Project)	110,104
	5329-008 (Project)	34,920
	5330-009 (Project)	53,276
TOTALS		803,514
R&R Budget - Other Personnel Funds Requested	5318-001 (Admin Core)	24,946
	5323-001 (Core)	157,114
	5324-002 (Core)	575,676
	5325-001 (Project)	69,544
	5327-002 (Project)	29,007
	5328-003 (Project)	70,422
	5321-004 (Project)	96,876
	5322-005 (Project)	32,111

	5319-006 (Project)	77,674
	5320-007 (Project)	102,730
	5329-008 (Project)	39,676
	5330-009 (Project)	176,500
TOTALS		1,452,276
R&R Budget - Section A & B Total Salary, Wages and Fringe Benefits (A+B)	5318-001 (Admin Core)	87,693
	5323-001 (Core)	199,246
	5324-002 (Core)	720,394
	5325-001 (Project)	80,624
	5327-002 (Project)	134,186
	5328-003 (Project)	90,092
	5321-004 (Project)	128,448
	5322-005 (Project)	89,961
	5319-006 (Project)	207,939
	5320-007 (Project)	212,835
	5329-008 (Project)	74,596
	5330-009 (Project)	229,776
TOTALS		2,255,790
R&R Budget - Section C Total Equipment	5318-001 (Admin Core)	0
	5323-001 (Core)	0
	5324-002 (Core)	0
	5325-001 (Project)	0
	5327-002 (Project)	0

	5328-003 (Project)	0
	5321-004 (Project)	0
	5322-005 (Project)	0
	5319-006 (Project)	0
	5320-007 (Project)	0
	5329-008 (Project)	0
	5330-009 (Project)	0
TOTALS		0
R&R Budget - Domestic Travel	5318-001 (Admin Core)	48 808
	5323-001 (Core)	2,500
	5324-002 (Core)	5,000
	5325-001 (Project)	3,000
	5327-002 (Project)	6 000
	5328-003 (Project)	4 000
	5321-004 (Project)	3,000
	5322-005 (Project)	3,000
	5319-006 (Project)	6,500
	5320-007 (Project)	3,000
	5329-008 (Project)	0
	5330-009 (Project)	10,000
TOTALS		94,808
R&R Budget - Foreign Travel	5318-001 (Admin Core)	0
	5323-001 (Core)	0
	5324-002 (Core)	0

	5325-001 (Project)	0
	5327-002 (Project)	0
	5328-003 (Project)	0
	5321-004 (Project)	0
	5322-005 (Project)	0
	5319-006 (Project)	0
	5320-007 (Project)	0
	5329-008 (Project)	0
	5330-009 (Project)	0
TOTALS		0
R&R Budget - Section D Total Travel	5318-001 (Admin Core)	48,808
	5323-001 (Core)	2,500
	5324-002 (Core)	5 000
	5325-001 (Project)	3 000
	5327-002 (Project)	6,000
	5328-003 (Project)	4,000
	5321-004 (Project)	3,000
	5322-005 (Project)	3,000
	5319-006 (Project)	6,500
	5320-007 (Project)	3,000
	5329-008 (Project)	0
	5330-009 (Project)	10,000
TOTALS		94,808
R&R Budget - Tuition/Fees/Health Insurance	5318-001 (Admin Core)	0

	5323-001 (Core)	0
	5324-002 (Core)	0
	5325-001 (Project)	0
	5327-002 (Project)	0
	5328-003 (Project)	0
	5321-004 (Project)	10,000
	5322-005 (Project)	0
	5319-006 (Project)	0
	5320-007 (Project)	0
	5329-008 (Project)	6,000
	5330-009 (Project)	0
TOTALS		16,000
R&R Budget - Stipends	5318-001 (Admin Core)	0
	5323-001 (Core)	0
	5324-002 (Core)	0
	5325-001 (Project)	0
	5327-002 (Project)	0
	5328-003 (Project)	0
	5321-004 (Project)	0
	5322-005 (Project)	0
	5319-006 (Project)	0
	5320-007 (Project)	0
	5329-008 (Project)	0
	5330-009 (Project)	0

TOTALS		0
R&R Budget - Trainee Travel	5318-001 (Admin Core)	0
	5323-001 (Core)	0
	5324-002 (Core)	0
	5325-001 (Project)	0
	5327-002 (Project)	0
	5328-003 (Project)	0
	5321-004 (Project)	0
	5322-005 (Project)	0
	5319-006 (Project)	0
	5320-007 (Project)	0
	5329-008 (Project)	0
	5330-009 (Project)	0
TOTALS		0
R&R Budget - Subsistence	5318-001 (Admin Core)	0
	5323-001 (Core)	0
	5324-002 (Core)	0
	5325-001 (Project)	0
	5327-002 (Project)	0
	5328-003 (Project)	0
	5321-004 (Project)	0
	5322-005 (Project)	0
	5319-006 (Project)	0
	5320-007 (Project)	0

	5329-008 (Project)	0
	5330-009 (Project)	0
TOTALS		0
R&R Budget - Other Participants/Trainee Support Costs	5318-001 (Admin Core)	0
	5323-001 (Core)	0
	5324-002 (Core)	0
	5325-001 (Project)	0
	5327-002 (Project)	0
	5328-003 (Project)	0
	5321-004 (Project)	0
	5322-005 (Project)	0
	5319-006 (Project)	0
	5320-007 (Project)	0
	5329-008 (Project)	0
	5330-009 (Project)	0
TOTALS		0
R&R Budget - Section E Total Participants/Trainee Support Costs	5318-001 (Admin Core)	0
	5323-001 (Core)	0
	5324-002 (Core)	0
	5325-001 (Project)	0
	5327-002 (Project)	0
	5328-003 (Project)	0
	5321-004 (Project)	10,000
	5322-005 (Project)	0

	5319-006 (Project)	0
	5320-007 (Project)	0
	5329-008 (Project)	6,000
	5330-009 (Project)	0
TOTALS		16,000
R&R Budget - Materials and Supplies	5318-001 (Admin Core)	750
	5323-001 (Core)	200,418
	5324-002 (Core)	318,021
	5325-001 (Project)	79,208
	5327-002 (Project)	125,764
	5328-003 (Project)	46,408
	5321-004 (Project)	117,200
	5322-005 (Project)	16,319
	5319-006 (Project)	40,000
	5320-007 (Project)	39,888
	5329-008 (Project)	29,495
	5330-009 (Project)	114,701
TOTALS		1,128,172
R&R Budget - Publication Costs	5318-001 (Admin Core)	0
	5323-001 (Core)	0
	5324-002 (Core)	0
	5325-001 (Project)	2,000
	5327-002 (Project)	2,000
	5328-003 (Project)	0

	5321-004 (Project)	0
	5322-005 (Project)	750
	5319-006 (Project)	2,000
	5320-007 (Project)	2,000
	5329-008 (Project)	0
	5330-009 (Project)	0
TOTALS		8,750
R&R Budget - Consultant Services	5318-001 (Admin Core)	12,500
	5323-001 (Core)	0
	5324-002 (Core)	0
	5325-001 (Project)	0
	5327-002 (Project)	0
	5328-003 (Project)	0
	5321-004 (Project)	0
	5322-005 (Project)	0
	5319-006 (Project)	0
	5320-007 (Project)	0
	5329-008 (Project)	0
	5330-009 (Project)	0
TOTALS		12,500
R&R Budget - ADP/Computer Services	5318-001 (Admin Core)	0
	5323-001 (Core)	0
	5324-002 (Core)	0
	5325-001 (Project)	0

	5327-002 (Project)	0
	5328-003 (Project)	0
	5321-004 (Project)	0
	5322-005 (Project)	0
	5319-006 (Project)	0
	5320-007 (Project)	0
	5329-008 (Project)	0
	5330-009 (Project)	0
TOTALS		0
R&R Budget - Subawards/Consortium/Contractual Costs	5318-001 (Admin Core)	0
	5323-001 (Core)	0
	5324-002 (Core)	0
	5325-001 (Project)	0
	5327-002 (Project)	0
	5328-003 (Project)	0
	5321-004 (Project)	0
	5322-005 (Project)	0
	5319-006 (Project)	0
	5320-007 (Project)	0
	5329-008 (Project)	0
	5330-009 (Project)	0
TOTALS		0
R&R Budget - Equipment or Facility Rental User Fees	5318-001 (Admin Core)	0
	5323-001 (Core)	0

	5324-002 (Core)	0
	5325-001 (Project)	0
	5327-002 (Project)	0
	5328-003 (Project)	0
	5321-004 (Project)	0
	5322-005 (Project)	0
	5319-006 (Project)	0
	5320-007 (Project)	0
	5329-008 (Project)	0
	5330-009 (Project)	0
TOTALS		0
R&R Budget - Alterations and Renovations	5318-001 (Admin Core)	0
	5323-001 (Core)	0
	5324-002 (Core)	0
	5325-001 (Project)	0
	5327-002 (Project)	0
	5328-003 (Project)	0
	5321-004 (Project)	0
	5322-005 (Project)	0
	5319-006 (Project)	0
	5320-007 (Project)	0
	5329-008 (Project)	0
	5330-009 (Project)	0
TOTALS		0

R&R Budget - Other Direct Cost 1	5318-001 (Admin Core)	4,655
	5323-001 (Core)	99,000
	5324-002 (Core)	3,250
	5325-001 (Project)	2,500
	5327-002 (Project)	15,000
	5328-003 (Project)	5,000
	5321-004 (Project)	26,000
	5322-005 (Project)	4,678
	5319-006 (Project)	13,600
	5320-007 (Project)	0
	5329-008 (Project)	40,000
	5330-009 (Project)	8,000
TOTALS		221,683
R&R Budget - Other Direct Cost 2	5318-001 (Admin Core)	12,500
	5323-001 (Core)	53,280
	5324-002 (Core)	0
	5325-001 (Project)	2,000
	5327-002 (Project)	13,000
	5328-003 (Project)	4,500
	5321-004 (Project)	12,130
	5322-005 (Project)	1,250
	5319-006 (Project)	7,000
	5320-007 (Project)	0
	5329-008 (Project)	0

	5330-009 (Project)	0
TOTALS		105,660
R&R Budget - Other Direct Cost 3	5318-001 (Admin Core)	1,750
	5323-001 (Core)	0
	5324-002 (Core)	0
	5325-001 (Project)	3,000
	5327-002 (Project)	8,421
	5328-003 (Project)	0
	5321-004 (Project)	3,177
	5322-005 (Project)	0
	5319-006 (Project)	60,000
	5320-007 (Project)	0
	5329-008 (Project)	0
	5330-009 (Project)	0
TOTALS		76,348
R&R Budget - Section F Total Other Direct Cost	5318-001 (Admin Core)	32,155
	5323-001 (Core)	352,698
	5324-002 (Core)	321,271
	5325-001 (Project)	88,708
	5327-002 (Project)	164,185
	5328-003 (Project)	55,908
	5321-004 (Project)	158,507
	5322-005 (Project)	22,997
	5319-006 (Project)	122,600

	5320-007 (Project)	41,888
	5329-008 (Project)	69,495
	5330-009 (Project)	122,701
TOTALS		1,553,113
R&R Budget - Section G Total Direct Cost (A thru F)	5318-001 (Admin Core)	168,656
	5323-001 (Core)	554,444
	5324-002 (Core)	1,046,665
	5325-001 (Project)	172,332
	5327-002 (Project)	304,371
	5328-003 (Project)	150,000
	5321-004 (Project)	299,955
	5322-005 (Project)	115,958
	5319-006 (Project)	337 039
	5320-007 (Project)	257 723
	5329-008 (Project)	150,091
	5330-009 (Project)	362,477
TOTALS		3,919,711
R&R Budget - Section H Indirect Costs	5318-001 (Admin Core)	79,268
	5323-001 (Core)	407,765
	5324-002 (Core)	1,281,750
	5325-001 (Project)	90,474
	5327-002 (Project)	158,273
	5328-003 (Project)	78,000
	5321-004 (Project)	217,466

	5322-005 (Project)	54,500
	5319-006 (Project)	222,154
	5320-007 (Project)	146,902
	5329-008 (Project)	79,971
	5330-009 (Project)	414,580
TOTALS		3,231,103
R&R Budget - Section I Total Direct and Indirect Costs (G +H)	5318-001 (Admin Core)	247,924
	5323-001 (Core)	962,209
	5324-002 (Core)	2,328 415
	5325-001 (Project)	262 806
	5327-002 (Project)	462,644
	5328-003 (Project)	228,000
	5321-004 (Project)	517 421
	5322-005 (Project)	170 458
	5319-006 (Project)	559,193
	5320-007 (Project)	404,625
	5329-008 (Project)	230,062
	5330-009 (Project)	777,057
TOTALS		7,150,814

A. COMPONENT COVER PAGE

Project Title: Administrative Core - Core A

Component Project Lead Information:

Whitley, Richard J.

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The Administrative Core of the Antiviral Drug Discovery and Development Center (AD3C) will provide a key role in leadership, communication, coordination and oversight of the projects and cores, and stimulate collaboration and synergy between the projects. Operationally, it is in charge of fiscal and contractual management of the center and will plan and implement activities, such as meetings of the Executive Committee (EC), External Scientific Advisory Board (EAB), and an annual meeting of all projects and cores. In addition, it will manage the inter-institutional cooperative agreements. The core is also responsible for managing the solicitation and review of Supplemental Research Projects applications, such as those for additional product development and support for IND-enabling studies. Finally, the core will facilitate dissemination of progress and discoveries to the public. The broad objectives of the core are thus as follows:

1. Providing programmatic and administrative leadership
    a Make decisions, specifically "go versus no-go" decisions per discussions with the EC
    b. Track and encourage research productivity
    c. Promote interactions and collaboration between projects and cores, in particular to facilitate overarching synergy to pursue broad-spectrum antivirals
    d Monitor the direction and overall priorities of the Center
    e Directly interface with NIH staff

2. Fiscal and administrative management of the center
    a. Finances: oversee expenditures, budget information, fiscal reports
    b Manage contracts and the consortium agreement
    c. Establish and monitor compliance with federal and NIH regulations

3. Develop, support and monitor progress of projects
    a. Manage projects by having regular conference calls and in-person meetings
    b. Organize quarterly project reviews (face to face or teleconference) by the EC
    c. Monitor overall Center research quality and progress annually by the EAB
    d. Solicit additional regulatory guidance on an as-needed basis
    e. Assist with identification and management of intellectual property developed by projects

4. Stimulate collaboration and synergy
    a. Identify potential areas or topics of collaborations between projects and cores
    b. Provide and facilitate access to resources needed in the projects
    c. Ensure that active hits in one project with potential against other viruses are evaluated in other projects
    d. Set up a data sharing and project tracking website

5. Facilitate meetings
    a. Host monthly conference calls of project teams
    b. Organize conference call-based and in-person meetings of the project and core PIs (EC)
    c.Organize and implement Annual face to face meeting with all involved personnel, including the EAB
    d.Facilitate consulting and other scientific and professional meetings
    e. Attend the annual CETR Program meeting and reverse site visit

6. Manage Supplemental Research Projects applications
    a. Solicit proposals
    b. Organize scientific and programmatic review of proposals

7. Outreach to the public
    a. Set up and maintain a website
    b. Write press releases
    c. Publish newsletters/ebriefs
    d Help with scientific publications

We firmly believe that a strong Administrative Core will be crucial to the success of the AD3C. We have aggregated projects led by leading virologists in the country and are confident that the Administrative Core will facilitate communication and collaboration between the PIs and the cores. Accomplishing the objectives set out above will ensure focus and synergy among the projects, accelerating the development of new, potentially broad-spectrum therapeutics for (re-)emerging infections of flaviviruses, coronaviruses, alphaviruses and influenza.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded. B2 Core A.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

In the upcoming reporting period, Year 3 of the AD3C grant, the Administrative Core plans to continue its leadership in ensuring that the operations and activities of AD3C get executed as efficiently as possible. To accomplish this, it will focus on the following activities within the listed goals:

1. Providing programmatic and administrative leadership
   a We will continue our monthly conference calls with research updates of the Projects and Cores, with associated minutes and metric-tracking The bi-weekly Core update meeting will also continue, as this has proven to be a very effective way of communicating action items and status reports for all of the Projects In addition, small group meetings will be set up between the Projects and Cores to cover technical details that warrant a detailed discussion that does not need to include all members of the Executive Committee.

2. Fiscal and administrative management of the center
   a Core staff will continue to provide guidance on the transition to new NIH formats for biosketches and other relevant NIH forms, and will work with sites to ensure that they are aware of any other federal regulations that are announced and related to the grant, particularly those that apply to sub-recipient awards Financial accounts and invoice submissions will be tracked closely and budget updates will continue to be provided to sites on a regular basis as effort on the projects continues to advance.

3. Develop, support and monitor progress of projects
   a. As mentioned under Item 1, the monthly conference calls have proven to be very efficient in monitoring progress in the projects and cores. Based on suggestions from the External Advisory Board following their annual review in October, these calls will be designed in the coming year to be less general and more focused on specific problems and solutions. In addition, we plan on having a mid-year face to face meeting with the Executive Committee members at the International Conference for Antiviral Research (ICAR), to be held in April 2016 in La Jolla, CA.

4. Stimulate collaboration and synergy
   a. Cores B and C continue to maintain a database that can be used to share activities of all tested compounds against the various viruses and assays, to quickly identify compounds with broad-spectrum efficacy. In addition, in the monthly conference calls, areas of collaboration and materials of use to multiple projects continue to be routinely identified and offered by the personnel involved As more potent and chemically tractable compounds are being identified through Core C's optimization efforts, we expect to see an increase of cross-virus activity testing in the next project period.

5. Facilitate meetings
   a. The Administrative Core personnel will continue to host the monthly teleconference and ad hoc conferences to address specific concerns. In addition, one of the project investigators has arranged with ICAR to reserve a time block for AD3C investigators to present at the large annual meeting in April 2016 The Administrative Core will assist with coordinating the travel and presentations In addition, it will organize the mid-year Executive Committee face-to-face meeting at the ICAR meeting in La Jolla, CA. Finally, the core staff will organize the third annual AD3C meeting with all personnel involved, in Birmingham, AL. We will once again host the External Advisory Board during the annual AD3C meeting, to give them the most up-to-date information about the research progress prior to their evaluation of the program We will expand the annual meeting to a full 2 days, to have time for in depth detailed discussions between Project and Core personnel

6. Manage Supplemental Research Project applications
   a If the CETR program provides, as mentioned in the RFA, supplemental funds for additional research projects, the Administrative Core will take charge of soliciting proposals and organizing their scientific and programmatic review

7. Outreach to the public
   a. The Admin Core will maintain and update www.uab.edu/ad3c as pertinent.

The major activities of Core A along with their specific objectives are described in bulleted form below, with a short description of the current status. Overall, Core A has met its objectives in Yr2, providing leadership and support to allow the other Cores and Projects achieve their respective goals and be successful.

1. **Providing programmatic and administrative leadership**
   a. Make decisions, specifically "go versus no-go" decisions per discussions with the EC
      We continued the practice of meeting with the EC and additional personnel on a monthly basis, via teleconference, to get an update on each of the projects and their interaction with the cores.
   b. Track and encourage research productivity
      The teleconferences mentioned above were summarized in minutes distributed back to the AD3C participants. Metrics such as publications and IP applications are actively being tracked.
   c. Promote interactions and collaboration between projects and cores
      The projects and cores have been collaborating heavily, with interactions facilitated by the Administrative Core as well as initiated by project and core leaders and personnel themselves to discuss ad-hoc technical issues. As started last year, hit compounds coming out of one project are being tested in others, although we expect this to accelerate as we find more potent and chemically attractive lead compounds.
   d. Monitor the direction and overall priorities of the center
      As in Yr1, the EAB met after the annual AD3C meeting and provided a report to the Administrative Core, attached to the overall CETR component of this progress report.
   e. Directly interface with NIH staff
      We have continued to communicate with NIAID program staff on several occasions and distributed pertinent information to the other research sites.
2. **Fiscal and administrative management of the center**
   a. Finances: oversee expenditures, budget information, fiscal reports
      The Administrative Core has provided payment and tracking information to sites and provided guidance on current Year 2 budgeting as well as projections for Year 3. Staff also worked with three of the projects to obtain carryforward funding needed to complete, in particular, equipment purchases delayed by agreement and weather-related delays late in Year 1.
   b. Manage contracts and the consortium agreement
      An institutional consortium agreement was finally finalized early in the 2015 calendar year, allowing the Year 2 subawards to be executed early in the grant year. Also, as noted in 2a. carryforward amendments were requested and approved for 3 projects.
   c. Establish and monitor compliance with federal and NIH regulations
      The Administrative Core staff continues to maintain a contact list which includes key research, administrative and financial personnel from all of the AD3C sites and is in regular communication with them regarding all AD3C activities and regulatory and financial requirements under the award including forms such as the biosketch, Uniform Guidance updates and those related to publications and data sharing. The Business Officer, Mary Wyatt Bowers, continues to work closely with UAB's Office of Sponsored Program and Grant Accounting as well as with similar offices in the participating institutions to ensure submission of required documents and compliance with federal policies.
3. **Develop, support and monitor progress of projects**
   a. Manage projects by having regular conference calls and in-person meetings
      As noted above, project teams and the EC had monthly conference calls with progress being tracked and to-do-items clearly delineated by preparing and distributing minutes by the Associate Director. In addition, there is an in-person meeting every 2 weeks between all Cores at Southern Research, along with Investigators from Project 4
   b. Organize quarterly project reviews (face-to-face or teleconference) by the EC
      As elaborated above, the EC was present at the monthly project team conference calls and received updates at that time. We will continue to monitor if separate quarterly meetings are needed in the future, but at this point, the monthly update meetings serve the goal of informing the EC of progress and obtaining their input on decisions to be made.
   c. Monitor overall Center research quality and progress annually by the EAB
      The EAB met for the second time at the annual meeting in October of 2015 and they provided a report with advice on a high, project portfolio level.

d. Solicit additional regulatory guidance on an as-needed basis

Projects are in such an early stage at this point that additional regulatory guidance has not been needed.

e. Assist with identification and management of Intellectual Property developed by projects

As described in the consortium agreement, novel chemical scaffolds with potent antiviral activity will be protected, using Southern Research as the lead institution since they have the most expertise in this area. At this point we do not have chemical series with the appropriate parameters that needs to be protected, but we expect that to change in the next project periods.

4. Stimulate collaboration and synergy

a. Identify potential areas or topics of collaborations between projects and cores

As mentioned above, there are already examples of compounds being tested in one project for antiviral activity being shared with other projects to test in their assays. This will continue to ramp up as new chemical scaffolds are being identified.

b. Provide and facilitate access to resources needed in the projects

The Admin Core facilitated transfer of non-used funds from Core A to Core B, since the screening library was expanded to cover more chemical space.

c. Ensure that active hits in one project with potential against other viruses are evaluated in other projects

As mentioned earlier, this is expected to increase in the next project periods as lead compounds are being identified.

d. Set up a data sharing and project tracking website

We continue to use the Enterprise Content Management software "Documentum CenterStage". All the AD3C and EAB members have access to this secured site via a login name and password. It contains meeting minutes and slide presentation files, and a limited database of antiviral activity of compounds to be tested. Core C maintains a specialized database with more advanced tools to query structures, antiviral activity and other compound parameters.

5. Facilitate meetings

a. Host monthly conference calls of project teams

An audio and web meeting service available through AT&T has been used by Administrative Core personnel to host the monthly project team meetings.

b. Organize conference call-based and in-person meetings of the project and core PIs (EC)

The EC has met during the annual AD3C meeting last October. We will convene again in April 2016, in conjunction with the International Conference for Antiviral Research, in La Jolla, CA.

c. Organize and implement Annual face to face meeting with all involved personnel, including the EAB

The Admin Core has hosted AD3C's second annual scientific meeting, in Birmingham, AL, on October 21-22, 2015, to discuss the status of each of the projects. Two of our EAB members also attended. Small group meetings were used to cover technical details of screening protocols and chemistry directions.

d. Facilitate consulting and other scientific and professional meetings

The projects are in an early phase of discovery and no consulting other than that received from the EAB has been required as of yet.

e. Attend the annual CETR Program meeting and reverse site visit

The Administrative Core facilitated the attendance of Drs. Everts, Suto, (b)(6) (b)(3)7 U S C § 8401 Baric (b)(6) (b)(3)7 and Prichard, in person at the reverse site visit, held November 19 2015, along with EAB member Dr. George Painter. Dr. Whitley and Ms. Bowers attended via conference call.

6. Manage Supplemental Research Projects applications

There has not yet been an RFA for Supplemental Research Projects from NIAID, so no activity to report.

7. Outreach to the public

a. Set up and maintain a website

The Administrative Core has set up a website: www.uab.edu/ad3c.

b. Write press releases

No press releases in this project period

c. Publish newsletters/ebriefs

The Administrative Core has not had a need yet to publish an electronic "ebrief".

d. Help with scientific publications

The Admin Core continues to ensure acknowledgement of grant support and submission to PubMed Central and PMCID requirements.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 063690705

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Richard		Whitley	MD	Project Lead	(b)(4) (b)(6)				18,330.00	5,517.00	23,847.00
2.		Maaike		Everts	PhD	Co-investigator					29,900.00	9,000.00	38,900.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **62,747.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	Program Manager, Program Coordinator	(b)(4)			18,520.00	6,426.00	24,946.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**24,946.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**87,693.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 063690705
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	48,808.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**48,808.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0 00
2. Stipends		0.00
3. Travel		0 00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 063690705

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

F. Other Direct Costs	Funds Requested ($)*
1 Materials and Supplies	750.00
2 Publication Costs	0 00
3. Consultant Services	12,500.00
4. ADP/Computer Services	0.00
5 Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Teleconference/Web meeting, website maintenance	4,655.00
9. Annual meeting expenses(space, refreshments, AV)	12,500.00
10. Copier, postage, shipping	1,750.00
Total Other Direct Costs	**32,155.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**168,656.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	47.0	168,656.00	79,268.00
		Total Indirect Costs	**79,268.00**

Cognizant Federal Agency

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**247,924.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Admin Core Budget Justification Yr 3 12-2015 final.pdf (Only attach one file.)

RESEARCH & RELATED Budget (F-K) (Funds Requested)

BUDGET JUSTIFICATION

Personnel

Richard J. Whitley, MD, PD/PI (b)(4) months: Dr. Whitley will continue to serve as the Program Director/Principal Investigator of AD3C, and as Director of the Administrative Core. Dr. Whitley is currently Professor of Pediatrics, Microbiology, Medicine and Neurosurgery, and holds the Loeb Chair in Pediatrics in the School of Medicine at the University of Alabama at Birmingham (UAB). He will continue to manage and provide guidance and oversight to the AD3C investigators, communicate regularly with the NIAID Program Officer, members of the EC and Scientific Advisory Board, and work closely with the Associate Administrator and staff to ensure that planned project milestones are met. He will also continue to moderate monthly teleconferences, lead the NIH reverse site visits, and the planned Center annual meeting which is expected to be held, as in the past two years, in October 2016.

Maaike Everts, PhD - Associate Administrative Director (b)(4) months,
Dr. Everts is an Associate Professor in the Department of Pediatrics, School of Medicine at the University of Alabama at Birmingham. She continues to service as the Associate Director of the Alabama Drug Discovery Alliance. She will continue to lead day to day research efforts of the AD3C, facilitating communication and interaction between project investigators and the cores, and serve as primary liaison between projects, providing research updates to the Director, Executive Committee and Scientific Advisory Board. She will also continue to work closely with the Program Director and Administrative Core staff to monitor the status of all projects and cores with respect to administrative, financial and regulatory aspects of the program.

Mary Wyatt Bowers, MA, (b)(4) months: Ms. Bowers will continue to oversee the financial administration of AD3C, and maintain responsibility for budgetary issues and invoicing, and coordinate with Sponsored Programs on subawards, and amendments. She will continue to aid Maaike Everts (see above) with meeting organization and planning, the annual noncompetitive renewal application and all other proposals related to AD3C funding. She also serves as the liaison with the UAB Office of Sponsored Programs, Grant Accounting, and Principal Investigators and institutions for matters related to financial and contractual agreements.

Sara Davis (b)(4) months, Ms. Davis is the program coordinator and administrative assistant to Dr. Rich Whitley. She will continue to assist Drs. Whitley and Everts with logistical aspects of meeting scheduling and organization as well as communications with external institutions and agencies and report preparations.

Consultants

Funds are budgeted for five members of the required external scientific advisory board to attend Center meetings and review activities of all projects. As projects enter year three, their continued guidance and oversight, and recommendations for the four projects remains of critical importance. The budget is based on estimated $2,500 reimbursement per advisor annually.

Supplies

Minimal funding is requested to provide for copier, postage, and office supplies needed to manage administrative activities of the large multi project program. Costs are estimated based on historical experience with similar multi-site project management.

Travel

Funds are requested for the following travel:

- Annual NIH reverse site visit expenses for the PD/PI, 1-2 project leaders and administrative staff in Rockville, MD.

- PD/PI, core personnel and/or Administrative Associate Director travel to provide technical assistance or oversight to one or more projects ($3,000)
- Executive committee meeting for PD/PIs and project leaders held approximately six months after Center Annual meeting.
- AD3C Center annual meeting held in Birmingham that includes PD/PIs, project leaders, postdocs, scientific advisory board members.
- Travel for project leaders and investigators to attend the annual International Conference on Antiviral Research (ICAR) to be held in LaJolla, CA on April 17-21, 2016. Based on discussions with the ICAR Program chair, who is also an AD3C investigator, a block of time has been set aside for the AD3C investigators to present their research. This meeting will replace the National CETR meeting which is no longer scheduled, per the November 2015 reverse site visit discussions.

Other Expenses

Teleconference/web meeting: Funds will continue to be used to cover the costs of monthly teleconferences and/or web meetings outlined in the administrative core plan to review and monitor projects and update project leaders on a continuous basis.

Computer Website design and maintenance: costs include continued website maintenance by a university professional to allow for communication and data sharing among investigators as well as providing a means for public access.

Publication/duplication costs: Funding is requested to cover costs related to publications and copying of draft progress reports, meeting minutes, and material used for annual or executive committee meetings. Costs are based on historical costs for meeting and reports used for a similar program.

Annual meeting expenses: funds are included to cover costs related to hosting a large Center meeting for investigators and scientific advisors. Based on suggestions following the recently completed annual meeting, it is expected that the meeting will be expanded to two full days in the coming year. The budget includes costs for meeting space, audiovisual services, incidental refreshments for attendees, and rental of items such as poster display boards.

A. COMPONENT COVER PAGE

Project Title: Project 1 1 Identification and Development of Anti-Flavivirus Lead Drug Candidates

Component Project Lead Information:

NELSON, JAY A

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Project 1 is designed to identify and characterize small molecule inhibitors of flaviviruses, a family of single stranded, positive sense RNA viruses that are associated with significant worldwide morbidity and mortality. This proposal builds on existing expertise in small molecule screening for DENV and is designed to identify small molecule compounds with the potential to be developed as antiviral agents. The initial screen in this proposal will focus on two medically relevant flaviviruses: dengue viruses (DENV) and West Nile virus (WNV). An existing screening platform will be adapted to screen multiple compound libraries, which include a high representation of nucleoside and nucleotide analogs, potentially compounds that have activity against multiple flaviviruses. If broad-spectrum leads with efficacy against multiple viruses can be identified, their further development will be emphasized. In order to enrich for potentially broadly acting compounds, we will focus on compounds that target one of the following important enzymatic activities of the flavivirus NS5 protein: the RNA-dependent RNA polymerase (RdRp), which is essential for replication of the viral RNA genome and the 2'-O-methyltransferase (2'-O-MTase), which is required for the virus to evade the host innate immune response. These activities are conserved among the flaviviruses, and similar activities are found in other virus families as well. The overall CETR proposal contains several projects focused on various virus families that are linked by a central screening facility and compound libraries. Therefore, the parallel screening strategies will maximize the likelihood of identifying broad-spectrum antiviral agents that may function across multiple virus families. The specific aims of Project 1 are:

Aim 1: Employ a validated HTS primary assay to screen novel drug libraries for antiviral compounds to identify novel inhibitors of flavivirus replication.
Rationale: The Southern Research Screening Core (Core B) has developed and validated cell-based, high-throughput assays for inhibitors of DENV and WNV induced cytopathic effect (CPE). Initial use of this, or similar, assays has already identified several compounds with antiviral activity. Therefore, this assay will be used to screen novel libraries that have not previously been extensively screened against human pathogens.
Experimental strategy: A CPE based assay will be used as a primary screen for compounds with anti-DENV or anti-WNV activity. Additionally, the WNV screen will be modified in order to allow the detection of compounds that inhibit the viral 2'-O-MTase, thereby sensitizing the virus to the actions of interferon and its effectors. Following the initial screen, "hits" will be evaluated in dose response and cytotoxicity assays in order to determine EC50, CC50, and selective indexes.

Aim 2: Characterize the antiviral activity of hit compounds
Rationale: Hit compounds will be further characterized with regard to efficacy and mechanism of action. The primary screen will potentially identify compounds that inhibit any of the stages of the viral replication cycle, therefore, secondary experiments are designed to elucidate the stage at which individual compounds act. Additionally, we will also characterize the compounds with regard to breadth of activity against other viruses, and examine the potential for evolution of compound-resistant mutants.
Experimental strategy: We will initially test compounds against sub-genomic viral replicons, which will identify compounds that do not function through affecting viral entry or egress, allowing us to focus on inhibitors of translation, protein processing, or RNA replication. We will also identify compounds that function through inhibition of the 2'O MTase, as well as compounds that act non-specifically through induction of interferon or other innate pathways. Compounds will also be evaluated in viral growth assays in order to evaluate the their effect on inhibition of production of infectious progeny virus. Additionally, we will analyze compound effects against multiple viruses and in multiple cell types. Finally, we will test the ability of the virus to develop resistance to individual compounds, as well as characterize any such mutants.

Aim 3: Chemical optimization and in vivo efficacy of lead compounds in animal models of West Nile and Dengue infection.
Rationale: Hit compounds identified and characterized above will be optimized to increase efficacy, selectivity, and bioavailability. These compounds will progress to testing in mouse models of infection.
Experimental strategy: Specific compounds and scaffolds will be triaged by the Medicinal Chemistry and Lead Development Core (MCLDC). Compounds with appropriate pharmacokinetic properties will be tested for prophylactic and therapeutic effects in mouse models of WNV and DENV infection.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Project 1 Nelson B.2.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

B.6 Plans for next year. As stated above, we have recently received 10 additional compounds that will be examined in a manner similar to that described above. We also expect to receive additional hits from the screen as they become available, and to begin to work with the medicinal chemistry core to analyze compound analogs to elucidate structure-activity relationships In addition to the experiments used for secondary screening described above, compounds will also be analyzed with additional assays described in the proposal in order to prioritize candidates for further pharmacokinetic analysis and in vivo activity studies. In order to elucidate mechanism of action of active compounds, we will passage virus in the presence of individual compounds to determine if resistant mutants can be generated. Such mutants will be sequenced to determine mutations that confer resistance, potentially indicating the viral target of the active compound

PROJECT 1 – JAY NELSON – OREGON HEALTH & SCIENCE UNIVERSITY

B.2: What was accomplished under these goals?

Major activities and objectives: The major activities for this reporting period have been: HTS for small molecule inhibitors of DENV to be conducted by SRI Core B; construction of cell lines with inducible expression of IFIT1 to allow for development of HTS that will identify inhibitors of flavivirus 2'O'methyltransferase activity; and follow-up and characterization of compounds with anti-DENV and anti-WNV activity identified by SRI in preliminary screens. We have also collaborated with project 3 (alphaviruses) to analyze compounds identified by SRI as alphavirus inhibitors for anti-flavivirus activity.

Results and outcomes: As per the timeline described in the overall proposal, the HTS for DENV inhibitors has been completed by SRI Core B (see Core B summary section). After dose response analysis, approximately 45 compounds were considered hits for further analysis, and these hits were further prioritized based on medicinal chemists' analysis of compound structures. Additional amounts of 13 compounds were obtained by the screening core and sent to OHSU for further analysis. Results are shown for three of these compounds (SRI-00038424, -00038425, and -00038447). We initially assayed inhibition of viral protein expression by indirect immunofluorescence using an antibody specific for the DENV E protein. Serial dilutions of each compound were added to HEK293 cells at the same time as infection with DENV-2 (NGC, moi=1). Cells were stained at 48 h pi and images captured (Fig. 1). Fluorescent signal was also measured using a plate reader (Biotek Synergy HTX) and used to calculate IC50 values for protein inhibition (Fig 2A). As shown, compounds SRI-00038425 and SRI-00038447 show dose-dependent inhibition of protein expression. In contrast, compound SRI-00038424 showed a non-dose dependent partial inhibition across several concentrations. In parallel, toxicity was measured using an XTT-based assay (Roche) of cell viability. As shown, toxicity is low for all compounds (cell viability ≥80% of control at all concentrations; Fig 2B).

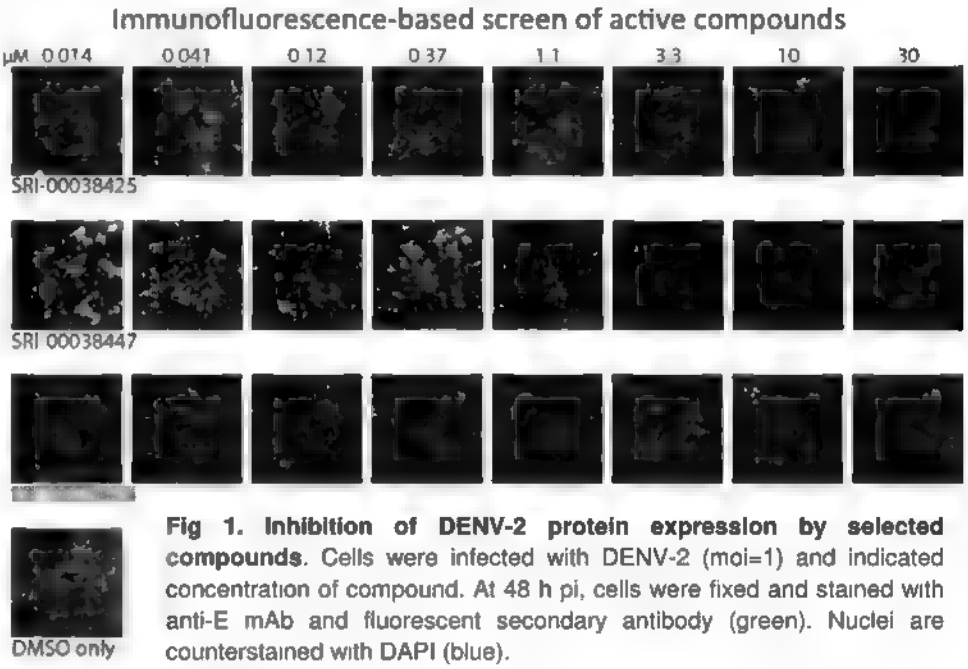
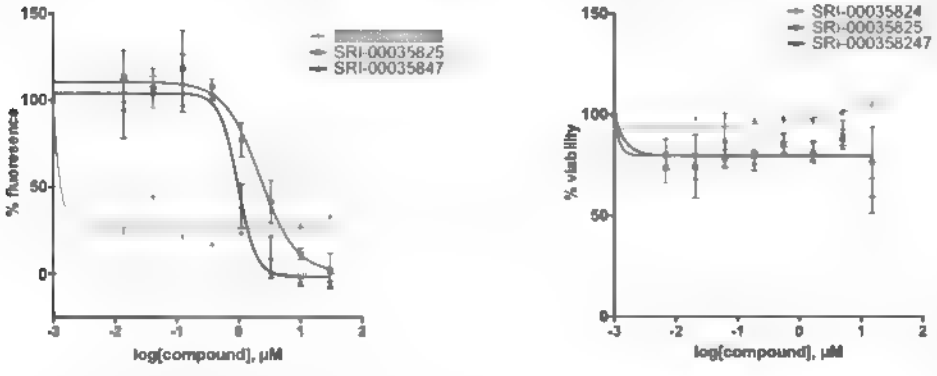


Fig 1. Inhibition of DENV-2 protein expression by selected compounds. Cells were infected with DENV-2 (moi=1) and indicated concentration of compound. At 48 h pi, cells were fixed and stained with anti-E mAb and fluorescent secondary antibody (green). Nuclei are counterstained with DAPI (blue).



SRI-00035825 IC_{50}: 2.2 μM
SRI-00035847 IC_{50}: 0.97 μM

Fig 2. Compound efficacy and toxicity. A. Alexa 488 (green) fluorescence intensity in each well (examples in Fig 1) was measured and used to calculate IC50 values using non-linear regression (GraphPad Prism). n=3 wells/ concentration. **B.** Percent viability at a range of compound concentrations was calculated using an XTT assay.

PROJECT 1 – JAY NELSON – OREGON HEALTH & SCIENCE UNIVERSITY

The most important measure of compound efficacy is, of course, its ability to reduce virus replication. We therefore tested these compounds in viral growth assays. Compounds were serially diluted and added to HEK293 cells with DENV-2. At 48 h pi, culture supernatants were collected and virus quantified by focus-forming assay (Fig 3). As shown, compound SRI-00038447 was most active, with and IC_{90} (i.e., the concentration at which 90% of viral replication is inhibited, or a 1 log10 reduction in titer) of 4.2 µM. Compound SRI-348425 showed some activity (IC_{90}=21), while compound SRI-00038424 was inactive in this assay.

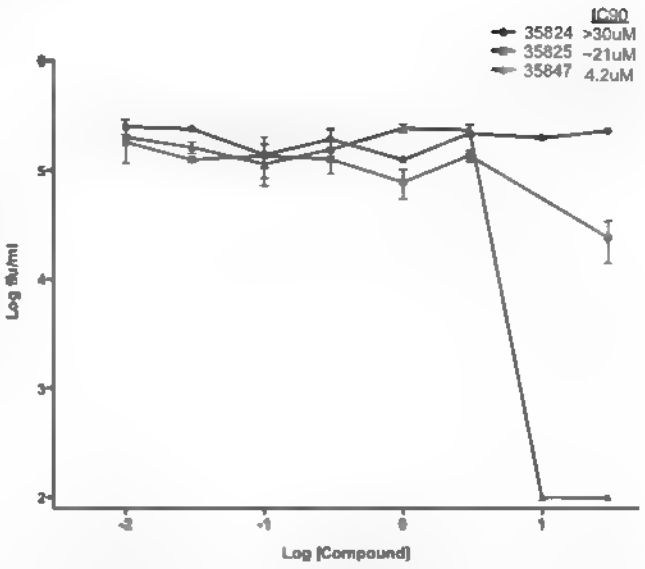


Fig 3. Inhibition of viral replication by selected compounds. Viral titers were determined by focus forming assay at 48 h pi. n=3 for each concentration.

In addition to secondary and tertiary screening of compounds, mouse embryonic fibroblasts (MEFs) with a genetic knockout of IFIT1 have been reconstituted with inducible IFIT1 expression by the Diamond lab. These cells have been provided to Core B and will be used in the subsequent screen for WNV inhibitors. Inhibitors of the viral 2'O-MTase, which is required to allow the virus to avoid the cellular innate immune response –in particular the action of IFIT1– are expected to function only in cells expressing IFIT1. Therefore, cells in the uninduced state (IFIT1 -/-) will be used as a counter screen to identify this specific class of inhibitor.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Oregon Health & Science University

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Jay		Nelson	PhD	Project Lead	(b)(4), (b)(6)				27,495.00	6,324.00	33,819.00
2.		Alec		Hirsch	PhD	Co-investigator					30,065.00	7,817.00	37,882.00
3.		(b)(6), (b)(3) 7 U.S.C. § 8401			PhD	Co-investigator					40,112.00	18,452.00	58,564.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **130,265.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	Staff Scientist, Research Associate	(b)(4)			58,491.00	19,183.00	77,674.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**77,674.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**207,939.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0969975150000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: Oregon Health & Science University

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	6,500.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**6,500.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0 00
2. Stipends		0.00
3. Travel		0 00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Oregon Health & Science University

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

F. Other Direct Costs	Funds Requested ($)*
1 Materials and Supplies	40,000.00
2 Publication Costs	2,000 00
3 Consultant Services	0.00
4. ADP/Computer Services	0.00
5 Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Animal charges (Per diem)	13,600.00
9. Microscopy, sequencing	7,000.00
10. M-Chem Core Services	60,000 00
Total Other Direct Costs	**122,600.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**337,039.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC On Campus	75.0	274,539.00	205,904.00
2. MTDC off campus	26.0	62,500.00	16,250.00
		Total Indirect Costs	**222,154.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**559,193.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Nelson_Project1_Budget_Justification_Year3_FINAL_MERGED.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Program Director/Principal Investigator (Last, First, Middle): Whitley, Richard J. (Project 1 – Nelson, Jay A.)

BUDGET JUSTIFICATION, YEAR 3:

Nelson- Anti-flavivirus drug discovery

PERSONNEL:

Jay Nelson, Ph.D., Principal Investigator: (b)(4) months, (b)(4) Dr. Nelson is a senior molecular virologist with over 180 papers and reviews on a variety of topics, including herpesviruses, retroviruses, and flaviviruses. The primary focus of Dr. Nelson's research over the years has centered on the molecular pathogenesis and immune response to viruses including herpesviruses, flaviviruses and retroviruses. Dr. Nelson's group has used molecular and animal model approaches over the past 30 years to characterize cytomegalovirus (CMV) and flavivirus replication. Dr. Nelson's group, in collaboration with Klaus Früh and Alec Hirsch, used functional genomic approaches to determine that cYes, a cellular Src kinase, is an important regulator of flavivirus maturation. We have also shown that capsid interaction with cYes alters tight junction function by targeting degradation of Claudin 1 by the lysosome and have shown that WNV regulates the unfolded protein response (UPR) through CHOP to block cellular apoptosis. This project is the result of a long-term collaboration between Drs. Früh and Hirsch to identify potential lead compounds that inhibit Dengue and WNV replication. Dr. Nelson will be responsible for the planning of experiments and oversight on progress for this grant, as well as for communication with other Project and Core leaders and dissemination of results.

Alec Hirsch, Ph.D., Co-Investigator: (b)(4) months (b)(4) Dr. Hirsch is an Assistant Scientist at the Vaccine and Gene Therapy Institute (VGTI) and will serve as Co-Investigator of the anti-flavivirus drug discovery project. Dr Hirsch has extensive experience with *in vitro* and *in vivo* models of flavivirus infection. He will be responsible for directing the investigation of the efficacy of compounds *in vitro* as well as *in vivo* in mouse models of viral infection. His duties will include coordination with other arms of this proposal, disseminating data sets produced during this project, and ensuring timely completion of the proposed work.

(b)(6) (b)(3) 7 U S C § 8401 **Ph.D., Co-Investigator:** (b)(4) months (b)(4) (b)(6) (b)(3) 7 U S C § 8401 received (b)(6) (b)(3) 7 Ph.D. in biomedical sciences from University of New Mexico School of Medicine in 2008, where (b)(6) studied entry and trafficking of human papilloma virus. Since (b)(6) (b)(3) 7 ime at the VGTI she has studied multiple aspects of flavivirus-host cell interactions, including identification and characterization of anti-flaviviral compounds (b)(6) will be responsible for directing lab personnel in conducting secondary and tertiary screens in conjunction with Dr. Hirsch, as well as supervising follow-up studies characterizing mechanisms of compound action. Additionally, (b)(6) (b)(3) 7 will be responsible for organizing and distributing data to other projects within the A3DC.

Meaghan Hancock, Ph.D., Staff Scientist 3: (b)(4) months (b)(4) Dr. Hancock is a molecular virologist working with Dr. Nelson the VGTI. She will be responsible for conducting secondary and tertiary screens as well as conducting follow-up studies characterizing mechanisms of compound action. Dr. Hancock will also assist in the *in vivo* studies to be conducted in later years of this project.

Christopher Parkins, M.S., Senior Research Associate: (b)(4) months (b)(4) Mr. Parkins will be responsible for producing WNV and DENV titered stocks for infection studies and assisting as needed with *in vitro* assays. Mr. Parkins will also be responsible for management of the AG129 mouse colony, infection of mice for *in vivo* studies, processing of animal samples, performing quantitative RT-PCR detection of virus in plasma and tissue samples from infected mice.

SUPPLIES:

Antibodies ($3,000/ yr Yr 1-5)

These are necessary for detection of WNV and DENV replication in focus forming assays, as well as detection of viral proteins by immunofluorescence and Western blotting. Additionally, specific antibodies recognizing DENV prM and E proteins are also used to mimic antibody-dependent enhancement of infection in the AG129 mouse model.

Plasticware ($5,000/ yr Yr 1-5)
Disposable plasticware will be required for cell and virus culture, DENV and WNV titration and virus isolation, and molecular biological work. This includes tissue culture dishes of myriad sizes and layouts, flasks, serological pipettes, disposable pipette tips, microfuge and centrifuge tubes, and disposable screw cap tubes of various sizes for sample storage.

Tissue Culture Supplies ($6,000/ yr Yr 1-5)
These will be required for all cell growth and maintenance as well as virus growth and titration and isolation from tissues. This includes cell culture growth media, animal serum, PBS, trypsin, sucrose, sorbitol, disposable sterilizing filters, antibiotics, and syringes.

qRT-PCR (Taqman) ($10,000/ yr Yr 1-5)
qRT-PCR will be used for the detection of both WNV and DENV in animal tissues and quantitation of viral RNA replication in culture. Reagents for virus detection include: Reverse transcription reagents, ABI Master mix containing Taq polymerase, virus-specific primers and TaqMan probes, 96-well optical plates and caps.

Surgical Supplies: ($2,500/ yr Yr 1-5)
Vacutainer blood tubes, needles, syringes and sterile plastic collection tubes and swabs required for obtaining blood samples and tissues, isoflurane for anesthesia; Alzet osmotic pumps for cases in which continuous delivery of compounds is to be examined.

Enzymes/ molecular biology supplies/ chemicals: ($5,000/ yr Yr 1-5)
PCR reagents for cloning of viral mutants, restriction enzymes, Western blotting and protein analysis supplies, buffers, acrylamide, agarose.

Toxicity assays: ($2,500/ yr Yr 1-5)
Celltiter Glo reagent (Promega) for determination of toxicity of individual compounds.

Mice purchase ($6,000/ yr Yr 1-5)
We expect that we will require approximately 350 of each strain for the experiments described in this proposal. We will maintain a colony of AG129 mice to provide mice for DENV experiments. Calculation of number of breeding cages and cages to maintain weaned mice to support proposed experiments (according to "Breeding Strategies for Maintaining Colonies of Laboratory Mice." Published 2007, The Jackson Laboratory) = 20 cages. We will purchase C57/Bl6 mice (3-4 weeks of age) from Jackson Laboratories ($16.40/animal) = approx. $6,000.

TRAVEL:
$6,500/year for Co-Investigators to attend an international meeting pertaining to antiviral therapeutics and vaccines directed against RNA virus infection and disease.

OTHER EXPENSES:
Mice per diem ($13,600/ yr Yr 1-5)
We expect that we will require approximately 350 of each strain for the experiments described in this proposal. We will maintain a colony of AG129 mice to provide mice for DENV experiments. Calculation of number of breeding cages and cages to maintain weaned mice to support proposed experiments (according to "Breeding Strategies for Maintaining Colonies of Laboratory Mice." Published 2007, The Jackson Laboratory) = 20 cages. At $1/cage/day per diem = $7,300 annually. Cage costs for weaned mice and housing during experiments at $3.50/cage/day per diem (approx. 2 months per cage) = $4,200. We will purchase C57/Bl6 mice (3-4 weeks of age) from Jackson Laboratories. Per diem cage costs for these mice at $3.50/cage/day per diem should total $2,100 (assuming a total of 1 month housing).

Microscopy: ($2,000/ yr Yr 1-5);
Quantitative fluorescence microscopy will be used in secondary screens to evaluate compound efficacy. Immunofluorescent staining will be performed at VGTI and plates read by the automated fluorescence

microscope at the Oregon Translational Research and Drug Development Institute (OTRADI). Cost is $50 for setup and $125/hour + 35% overhead.

Sequencing: ($5,000/ yr Yr 1-5)
At $500/sample, we will use deep sequencing to identify resistance mutations that arise due to compound treatment. We will sequence approximately 5 samples per year including both WNV and DENV isolates.

Publications ($2,000/ yr Yr 1-5)
For publication costs. We estimate 1-2 publications per year.

M-Chem Core Services ($60,000/ yr Yr 2-5)
M-Chem Core Services: ($60,000/yr, Years 2–5): Dr. Aaron Nilsen is Director of the OHSU Medicinal Chemistry Core facility (M-Chem Core). For this project, the M-Chem Core will design chemical biology experiments to help biological researchers investigate the mechanisms of action of small molecule antivirals. Additionally, the Core will synthesize analogs of small molecule antivirals for use in mechanism of action experiments. In terms of experience, the Core Director has more than 17 years of experience in chemical biology, medicinal chemistry, drug discovery and organic synthesis. Dr. Nilsen was the lead synthetic chemist on the multinational Medicines for Malaria Venture team that recently delivered a new quinolone-3-diarylether compound (ELQ-300) to the MMV for clinical development. Chemical reagents will be required to synthesize analogs including building blocks and solvents, which will be charged through the Core.

INDIRECT COSTS:
The bulk of the indirect costs ($205,904) are calculated at the rate of 75% (based on direct costs of $274,539) for work to be conducted at the OHSU West Campus. The remainder of the indirect costs ($16,250) are calculated at an F&A rate of 26%, which is the off-campus rate used for research projects at OHSU. It is OHSU's policy not to charge a significantly higher indirect cost rate on projects that transfer from outside entities, even though the work is done on-campus. Charging the full rate on this project would create a financial hardship in terms of achieving and completing the aims of the project. Therefore, a 26% modified total direct cost rate is used for that portion of the project ($62,500 direct costs: $60,000 for M-Chem core serves and $2,500 for travel) that will be conducted at the OHSU Medicinal Chemistry Core facility (transferred from the Portland VA Research Foundation, Inc., which is outside OHSU).

A. COMPONENT COVER PAGE

Project Title: Project 2 1 Inhibitors of Coronavirus Fidelity and Cap Methylation as Broadly Applicable Therapeutics

Component Project Lead Information:

(b (6) (b)(3) 7 U S C § 8401

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The overall goal of Project 2 is to identify inhibitors of two highly conserved CoV processes, replication fidelity and RNA capping, that are essential for SARS-CoV virulence and survival in vivo Multiple viral proteins and enzymatic activities are critical for these processes, including CoV 3'-to-5' exoribonuclease (fidelity, nsp14-ExoN) and 2'-O-methyltransferase (capping; nsp16-OMTase) activities. Consistent with the importance of these processes, we have shown that decreased replication fidelity and ablation of RNA capping through genetic inactivation of either ExoN or OMTase, respectively, results in replication competent viruses that are profoundly attenuated in vivo

Aim 1. To identify and develop inhibitors of CoV high-fidelity replication We will test the hypothesis that inhibitors of CoV high-fidelity replication will decrease viral fitness alone and in combination with RNA mutagens, and represent potent pan-CoV therapeutics. In part 1, we will identify ribonucleoside analogs that inhibit CoV replication, and define their mechanism of action High-throughput screening in part 2 will identify small-molecule inhibitors of CoV fidelity. In part 3 we will identify the viral protein targets of lead compounds, and determine their mechanism of fidelity impairment. In part 4, will we test highly efficacious compounds identified in parts 1 and 2 across the CoV family and viral platforms within this program.

Aim 2. To identify and develop inhibitors of CoV RNA capping activity. We hypothesize that small molecule inhibitors of essential CoV RNA capping components will profoundly increase CoV sensitivity to the host innate immune response through interferon-stimulated effectors In part 1 we will use targeted mutagenesis of known CoV capping components to define distinct mechanisms to increase CoV sensitivity to the host ISGs In part 2 we will examine the combined efficacy of known O-MTase inhibitors and type I IFN treatment against SARS-CoV, and perform a high-throughput screen for inhibitors of CoV RNA capping. In part 3 we will identify the viral protein targets and mechanism of action of lead compounds In part 4, lead compounds will be tested across the CoV family and specific viral platforms within this program

Aim 3. To chemically optimize and test the in vivo efficacy of CoV fidelity and RNA capping inhibitors We will test the hypothesis that inhibitors of CoV fidelity or RNA capping are highly attenuating in vivo and represent broadly effective CoV therapeutics. Compounds identified in Aims 1 and 2 will be chemically optimized for in vitro efficacy, selectivity, solubility, microsomal stability, and bioavailability at SR. Using these optimized compounds, in part 1 we will confirm the biological target(s) of lead fidelity and RNA capping inhibitors in vivo. In part 2 we will test the efficacy of lead compounds against mouse-adapted SARS-CoV in progressively stringent mouse models of acute and persistent human disease. Efficacy will be determined by monitoring respiratory function, morbidity and mortality, histology, and viral replication. In part 3 we will test for the development of drug resistance in vivo, and will determine the efficacy of lead compounds against MERS-CoV and other CoV family members.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Project 2 B2 Done 12.7 SD.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: Project 2 (b)(6), (b)(3).7 B4 Training IDPs Done 12.7.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Project 2: Inhibitors of Coronavirus Fidelity and Cap Methylation as Broadly Applicable Therapeutics (b)(6), (b)(3) 7 U S C § 8401 Ralph S. Baric)

B.6. Plans for Next Reporting Period
Plans for the next reporting period are based on continuation of the sections above as well as completion of screening for initial hits. Plans are presented as bullet points under specific topics. In vivo testing will begin with
SARS-CoV and then MERS-CoV will be evaluated once sufficient susceptible/esterase deficient mice can be
bred and in vitro (culture) testing with MHV, SARS-CoV and MERS-CoV. The focus of plans for the coming year will be on GS-5734

(Gilead) testing in animals (Baric) and for mechanism (b)(6) (b)(3)7 U.S.C. § 8401 as well as additional GS nucleoside analog testing in HAE (Baric) This will include participation in the IND submitted by Gilead for GS-5734. Other studies will move forward testing in vitro of SRI candidate SARS-CoV inhibitors in vitro against MERS and SARS (b)(6) (b)(3)7 for chemical modification (SRI) and for selected high value hits testing in HAE cells and possibly in vivo (Baric, SRI).

1. Testing of Gilead nucleoside analogs . (In collaboration with Gilead Sciences)

(b)(6) (b)(3)7 Lab
- Test the nsp12-RdRp F476L and V553L putative resistance mutations singly and combined in MHV, SARS-CoV and MERS-CoV isogenic recombinant viruses with GS-5734
- Test for cross resistance to mutagens and other nucleoside analogs of known and unknown mechanism
- Test hypothesis that resistance mutations are attenuated or less fit for replication.
- Determine if GS-5734 increases sensitivity of WT to other NAs or mutagens eg it functions to inhibit nsp14-ExoN proofreading.
- Test and establish cell lines that have better more consistent penetration of GS-5734 for rapid in vitro testing of WT and resistance mutants with GS-5734 These will also be the basis for testing of other identified nucleoside analogs.

Baric Lab
- Development of replication (MERS-CoV) and lethal (SARS-CoV, HKU5 MA, MERS-CoV) infection models for human coronavirus nucleoside analog testing in esterase deficient (CES -/-) mice
- Analysis of PK of GS-5734 in esterase deficient mice
- Determine appropriate drug concentration, route of administration and dosing regimen for GS-5734 for evaluating mice infected with MERS-CoV or SARS-CoV.

Testing for efficacy against SARS-CoV and MERS-CoV in vivo.
- Continue testing GS parent and prodrugs to determine EC90 values and to identify additional candidate compounds

2 SRI compounds (SRI, UAB (b)(6) and Baric Labs) Screening of >200K compounds has been completed for SARS-CoV using a CPE based assay. We have modified our plans to accelerate forward movement of candidate inhibitors.
- Testing of <20 compounds against MERS and SARS in vero cells using plaque assay for EC-50, EC-90 and log reduction measurements in vitro (b)(6) (b)(3)7 Data to SRI for determination of leads for PK and possible chemical modification (SRI)
- Optimization of nanoluc and FFL reporter virus assays for ongoing studies of modified or prodrugs

(b)(6) (b)(3)7 U.S.C. § 8401
- For high impact candidates, initiate passage for resistance, deep sequencing and possible mechanism.
- Testing in HAE cells of verified active compounds (Baric).
- Establish possible candidates for in vivo (mouse model) testing (Baric)

Project 2: Inhibitors of Coronavirus Fidelity and Cap Methylation as Broadly Applicable Therapeutics (b)(6), (b)(3) 7 U S C § 8401 Ralph S. Baric)

B.2. What was accomplished under these goals?

Summary of major activities, results and achievements. Significant and exciting progress was made on the project aims in both (b)(6) (b)(3) 7 U S C § 8401 and Baric Labs. Collaborations both with Southern Research Institute (SRI) and Gilead Sciences (GS).

B.2.a. We have made progress in collaboration with SRI in completing the initial CPE based screen of >200K compounds with initial hits indentifying compounds with activity against SARS-CoV. Downstream analysis will include both SARS-CoV and MERS-CoV in vitro and in human airway epithelial cells (HAE). This will be a main focus in the coming year, with selection for further chemical modification, confirmation and validation, testing for mechanism and forward movement into relevant in vivo models.

B.2.b. With GS, the major breakthrough and activities in this year focused initially on GS-5734, a nucleoside analog from GS, that was tested as a collaboration with both the (b)(6) (b)(3) 7 U S C § 8401 and Baric labs. GS-5734 is a prodrug of GS-441524, which was described as an active compound in last years progress report. Overall in this year we: **1)** showed high level in vitro activity of parent GS-441524 and prodrug GS-5734 against SARS-CoV, MERS-CoV and the model Group 2a CoV, murine hepatitis virus (MHV). **2)** demonstrated GS-5734 activity against SARS-CoV and MERS-CoV in an relevant human airway epithelium (HAE) cell model of infection and evaluated another 22 GS compounds in HAE; **3)** passaged MHV with GS-441524 active compound, established resistance and identified putative resistance mediating mutations in the CoV nsp12-RNA dependent RNA polymerase; and **4)** initiated development studies for in vivo testing for SARS-CoV and MERS-CoV inhibition in mouse models. ***In summary*** in this year we have tested 23 GS compounds in HAE and identified at least one highly active candidate inhibitor of multiple divergent group 2 CoVs, including SARS-CoV, MERS-CoV, and MHV, as well as relevant in vivo models and progress toward a mouse model for testing. Further we have a putative mechanism for resistance, pointing to a potential mechanism of action of the compound. GS-5734 has been shown to inhibit Ebola virus and has been used in humans in compassionate use basis with possible effectiveness. Thus the potential impact of our progress in this year is very high against a significant problem of continuing MERS-CoV outbreak in the middle east and globally.

1. In vitro testing of GS-441524 and prodrug GS-5734 demonstrates high activity and inhibition against SARS-CoV, MERS-CoV and MHV (b)(6) (b)(3) 7 U S C § 8401. From the previous year we demonstrated activity of the compound GS-441524 (Gilead Sciences) against MHV and SARS-CoV in vitro. This was the first demonstration of a nucleoside analog that was active against a WT coronavirus. We hypothesized that this resistance to nucleoside analogs acting as pol inhibitors, chain terminators or mutagens was due to the powerful proofreading activity of the nsp14-ExoN, in direct interaction with the nsp12-RNA dependent RNA polymerase. These protein functions are highly conserved across all CoVs. Thus we used the group 2A CoV, MHV for initial testing followed by testing with SARS-CoV and MERS-CoV. As reported last year, GS-441-524 showed activity with an EC50 of less than 1μM for both MHV and SARS-CoV in vitro. This was profoundly greater activity against WT and suggested a novel mechanism of action, potentially with inhibition of the proofreading ExoN. In this year we obtained multiple prodrugs of GS-441524 that allowed for better cell penetration and intracellular modification to the active triphosphate form of the compounds. More than 60% of the prodrugs showed enhanced EC50 and Log reduction compared with GS-441524 in vitro. Based on input from Gilead, we concentrated on the prodrug GS-1081 and the pure isomer of GS-1081, GS-5734. At the time, this was done with blind testing on our part. After testing, we were informed that GS-5734 showed profound inhibition of Ebola virus replication as well (Gilead Sciences, communication). GS-5734 is a phosphoramidate prodrug of GS-441524. The mechanism is not directly determined, but is proposed to be a polymerase inhibitor. In our testing the activity is at >20 fold increased by EC50 and 1000 fold greater by log reduction at concentrations <5μM compared with GS-441524.

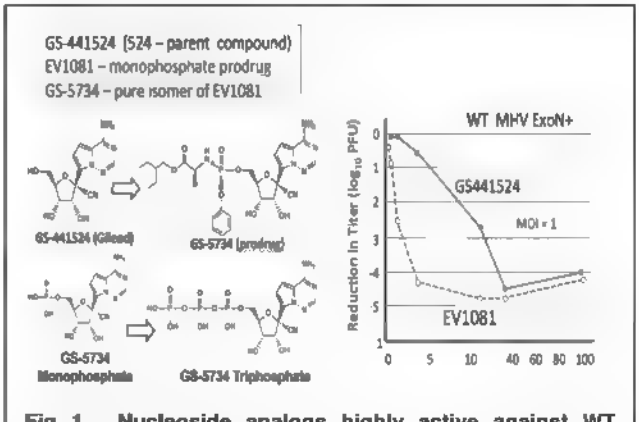


Fig 1. Nucleoside analogs highly active against WT (ExoN+) CoV replication. Parent (GS-441524)/prodrug (GS-5734) and pathway to active triphosphate activity are shown (left) In vitro against MHV is dramatically enhanced for EV1081/GS-5734 (right).

2. GS-5374 and related compounds inhibit SARS-CoV and MERS-CoV in differentiated HAE cultures (Baric Lab: (b)(6) (b)(3)7 (b)(6) (b (3):7 L S C § 8401 In vitro testing in Vero cells for SARS-CoV and MERS-CoV for nucleoside analogs, and particularly GS-441524 and GS-5734 is problematic for reproducibility and accuracy because of lack of nucleoside transporters. In addition it is critical to extend in vitro results in a relevant in vivo model. The Baric lab has established critical reporter viruses (MERS-RFP, MERS-Nano Luc, SARS-GFP, SARS-Nano Luc) and approaches using primary human lung-derived airway epithelial cell (HAE) cultures. The cells grow with an air-fluid interface in trans-wells and recapitulate the morphology of the human conducting airway and thus are a highly relevant "in vitro" model for testing. Using this system we have confirmed and extended in vitro results using GS-5734 and other prodrugs of GS-441524, showing consistent, high-level and reproducible inhibition of SARS-CoV and MERS-CoV replication. (Fig 2). In addition to GS-5734, we have also screened 23 additional Gilead parent and prodrugs against SARS-CoV and MERS-CoV, resulting in ten more drugs for downstream testing (Table 1).

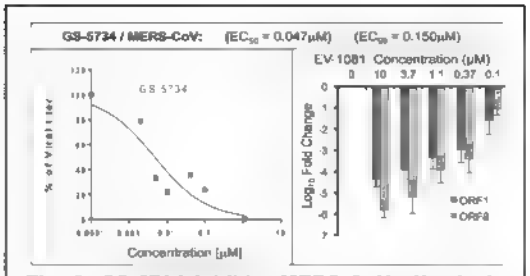


Fig. 2. GS-5734 inhibits MERS-CoV effectively in human airway epithelium cell culture. Log reduction of genome (ORF1) and subgenomic (ORF8) RNA is shown in right panel with increasing concentrations of EV-1081 (isomer mixture of GS 5734. Percent inhibition curves and EC50/90 calculation shown to the left panel

Table 1. Gilead compounds evaluated in MERS-CoV or SARS-CoV infected HAE.

Gilead Compound	Class of drug	Less of >1 log titer MERS-CoV (yield)*	Less of >1 log titer SARS-CoV (yield)*
EV-08*	Prodrug	Y	Y
EV-0806	Prodrug 004	N	N
EV-0804	Parent	N	N
EV-2248	Prodrug 524	Y	Y
EV-2284	Prodrug 524	Y	Y
EV-2263	Prodrug 524	Y	Y
EV-229	Prodrug 524	N	N
EV-0808	Parent 089	N	N
EV-2242	Prodrug 089	N	N
EV-08 8	pure isomer SP	Y	ND
EV-2302	Prodrug 089	N	N
EV-2304	Prodrug 089	N	N
EV-2305	Prodrug 089	N	N
EV-0804	524 parent	Y	Y
EV-2247	Prodrug 524	Y	Y
EV-226	Prodrug 524	N	Y
EV-2282	Prodrug 1826	N	Y
EV-2283	Prodrug 089	N	N
EV-2285	Prodrug	Y	N
EV-0***	Parent		Y
EV-0687	Prodrug	Y	Y
EV-0***	Parent		N
EV-0798	Prodrug	N	N

3. Resistance to GS-5734 and potential mechanism of action against CoVs. Because of consistency of in vitro testing and ability to work at BSL2, MHV model was used to test for resistance and initial testing for potential mechanism of action of GS-5734. Virus was passaged in presence of parent GS-441524. Resistance was very difficult to achieve, requiring multiple restarts of process and 23 passages. At P23, MHV demonstrated increased replication and viability at up to 30mM GS-441524. Sequencing identified two mutations resulting in substitutions in the nsp12-RdRp at V553L and F476L. The functions of these residues in Pol nsp12-pol function is not known. We introduced these mutations together in isogenic cloned MHV background with no other mutations. Testing of WT and the V553L/F476L mutant demonstrated that the two mutations were sufficient to reproduce the resistance phenotype against GS-441524 at least 37 fold at the EC50 and >100 fold at >10μM. As expected resistance was present to the prodrug GS-5734 as well, but was overcome at higher concentrations, likely due to better cell penetration and concentrations. In summary, resistance was difficult to achieve, but mapped to the nsp12-RdRp. This, along with activity of GS-5734 against WT, supports hypothesis that compound is acting as a direct pol inhibitor. We are testing these mutations in SARS and MERS-CoV backbones as well.

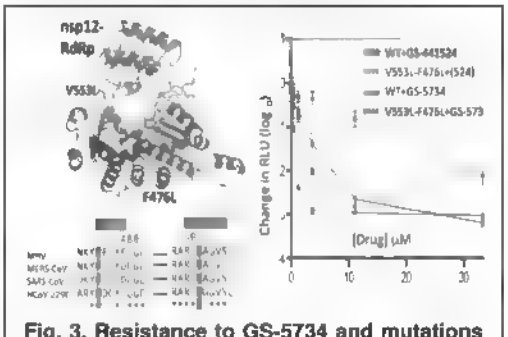


Fig. 3. Resistance to GS-5734 and mutations at conserved residues in the CoV nsp12-RdRp. Mutations identified in passage 23 (P23) of MHV with GS-441524 are modeled in the CoV nsp12-RdRp core pol domain. The residues are conserved across divergent α- and β-CoVs like MERS and SARS. Recombinant MHV with only the putative resistance mutations show resistance maps to the identified nsp12 residues, for both GS-441524 and GS-5374

4. Generation of mice models for in vivo testing of GS-5734 and related nucleoside analogs. (Baric Lab: Sheahan). A limitation to mouse testing of nucleoside analogs (nucs) is their inactivation by murine esterases. We are collaborating with GS to develop an esterase deficient mouse model (CES 1-/-) for lethal human coronavirus infection for downstream efficacy and safety testing. The Baric lab is working with GS to design a breeding workflow to generate CES 1-/- mice for in vivo drug testing with SARS- and MERS-CoV. CES 1 -/- mice and WT C57 BL/6 mice are equally susceptible to SARS-CoV infection (Fig. 4). In year 3 we will test the lethal mouse adapted CoV models (SARS-CoV and HKU5-S MA) in CES1-/-mice (10^5 PFU) providing a robust measure of GS-5734 inhibition. For MERS-CoV, drug will first be tested in hDPP4 transduced CES1-/- mice (replication only model). In parallel, we are also crossing the 288/330 humanized DPP4 mouse model to the CES1-/- mice to produce a lethal MERS-CoV mouse model for downstream testing of the GS and related drug portfolios.

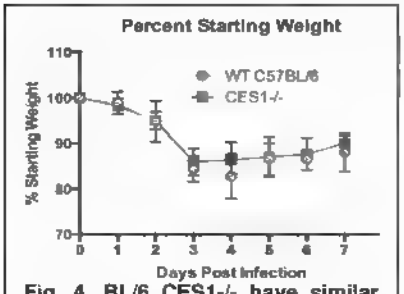


Fig. 4. BL/6 CES1-/- have similar phenotype as WT following SARS-CoV infection.

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

Graduate Students and Postdoctoral Fellows are active in the project at Vanderbilt (Smith, Sexton). Individual development plans (IDPs) are generated on an annual basis for all Graduate Students and Postdoctoral Fellows. They are used for defining key objectives and goals for progress and for review on at least an annual basis. For this project, the IDPs will review specific goals relevant to the project. For graduate students, these assist in analysis of progress in projects, and for postdoctoral fellows in addition they help in career development. For IDPs, both biosketches and CVs are appended, so that it is possible to use these as learning tools.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0044134560000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		(b)(6), (b)(3) 7 U S C § 8401			MD	Project Lead	(b)(4) (b)(6)				45,825.00	4,582.50	50,407.50
2.					PhD	Lab Manager					49,500.00	10,197.00	59,697.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **110,104.50**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b)(4)			35,078.40	8,629.29	43,707.69
1	Graduate Students				7,272 00	0 00	7,272 00
	Undergraduate Students						
	Secretarial/Clerical						
2	Research Assistant, Sr. Research Specialist				41,533.46	10,217.23	51,750.69
4	**Total Number Other Personnel**					**Total Other Personnel**	**102,730.38**
						Total Salary, Wages and Fringe Benefits (A+B)	**212,834.88**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0044134560000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0 00
2. Stipends		0.00
3. Travel		0 00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0044134560000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

F. Other Direct Costs	Funds Requested ($)*
1 Materials and Supplies	39,888 05
2 Publication Costs	2,000 00
3 Consultant Services	0.00
4. ADP/Computer Services	0.00
5 Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**41,888.05**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**257,722.93**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	57.0	257,722.93	146,902.07
		Total Indirect Costs	**146,902.07**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number) DHHS Steven Zuraf, 301-492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**404,625.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name Budget Justification U19 AI109680-03 Proj 2.pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION ([redacted], PROJECT YEAR 3)

PERSONNEL

[redacted] **Principal Investigator** [redacted] **months)** [redacted] will continue to serve as the Principal Investigator of Project 2. [redacted] experience studying coronavirus replication and replicase nonstructural protein functions. [redacted] has published extensively on reverse genetics, replication, and molecular biology of coronaviruses [redacted]

[redacted] **months)** [redacted] is a [redacted] BSL3 and SARS-CoV research program [redacted] has extensive experience with the design, recovery and analysis of SARS-CoV and MERS-CoV, [redacted] ontinues to be responsible for training, and oversight of experiments with SARS-CoV and MERS-CoV at BSL3. [redacted] is an authorized Select Agent user, and will coordinate all investigators and studies in the BSL3 and will directly perform portions of studies in Aim 1. Additionally, [redacted] will coordinate fragment synthesis with commercial companies, maintain genome fragments, and be responsible for experiments and training in the BSL3 laboratory.

[redacted] **months).** [redacted] is a [redacted] responsible for the discovery of the sensitivity of nsp14/ExoN- mutant viruses to RNA mutagens in both MHV and SARS-CoV. [redacted] designs and carries out experiments in Aim 1, with testing using the model MHV at BSL2 and SARS-CoV at BSL3. [redacted] is an authorized Select Agent user, and will be responsible for studies in Aim 1 of inhibition by different classes of nucleoside analogs.

[redacted] **months).** [redacted] s currently a [redacted] who has published experience working with emergent viruses. [redacted] will carry out experiments from Aim 1 that identify and test inhibitors that target the nsp12 RdRp, particularly in those that allow identification of resistance mutation in nsp12. [redacted] s an authorized Select Agent user. .

[redacted] **months)** [redacted] s a [redacted] specialist who has worked with [redacted] for over [redacted] During this time [redacted] publications, and has extensive experience in performing mutagenesis, and in engineering recombinant viruses using our reverse genetics system. [redacted] will carry out support experiments at BSL2 with mutagenesis, preparation of fragments, sequencing of MERS-CoV RNA and analysis of data. [redacted] will maintain critical chemical and reagent stocks as well.

[redacted] **months).** [redacted] is a research assistant 1 who has developed and optimized the luciferase containing MHV for initial testing of candidate inhibitors from Gilead Sciences and SRI. [redacted] will continue to perform initial testing and generation of virological growth curves and EC50 analysis of initial and optimized inhibitors. [redacted] also is responsible for cell culture preparation for all studies that will be performed at BSL3. [redacted] also performs initial mutagenesis of luciferase containing genome fragments for MHV, SARS-CoV and MERS-CoV for use in generating recombinant viruses. [redacted] will perform experiments, generate data, and analyze data in consultation with [redacted]

FRINGE BENEFITS: Fringe benefit calculations are derived from the current Vanderbilt University Medical Center guidelines.

LAB SUPPLIES ($39,888)

Cell Culture Supplies, Serum and Media ($10,000). A large amount of cell culture work is associated with the project, requiring media, serum, and culturing flasks. Consequently, funds are requested for media, serum, and related cell culture supplies to maintain Vero cells in culture, measure sensitivity to nucleoside analogs, and confirm target compounds. Reagents for the extended passage of SARS-CoV in the presence of nucleoside analogs and lead compounds to test for the development of resistance from Aim 1 will be required.

BSL3 Supplies, protective gear, disinfectants, decontamination ($20,000). All testing of nucleoside analogs, monitoring the development of drug resistance, and confirmation of lead compounds will be performed under strict BSL3 protocols. This will include extensive use of plasticware, tissue culture reagents, materials for plaque assays, and RNA isolation. BSL3 PPE (personal protective equipment) is also required for all work done at BSL3, as is an annual decontamination and complete recertification of the laboratory. This recertification includes required maintenance on any equipment within the BSL3. Regular delivery of CO_2 for the incubators is also needed. In addition, supplies for analysis of RNA and protein at BSL2 as well as materials for shipping of samples between UNC and Vanderbilt are required. Separate reagents are needed for analysis of SARS-CoV RNA at BSL2 because the RNA is a Select Agent.

Enzymes and Reagents ($9,888). Any potential resistant mutations will need to be reengineered into the SARS-CoV reverse genetics clone. Generating mutations within the plasmids carrying fragments of the viral genomes will require the enzymes and reagents necessary for these molecular biology protocols. Assembling recombinant SARS-CoV requires large amounts of highly expensive restriction enzymes (e.g., BsmBI, etc.) and large amounts of DNA ligase. DNA markers are needed for identifying appropriately sized bands and assembly intermediates and full-length DNA products in gels; a critical step during the assembly of full-length cDNA clones. In addition, high quality T7 RNA polymerase is needed for driving production of full-length RNA transcripts for electroporation into susceptible cells and for the subsequent recovery of recombinant viruses. Lead compounds identified in Aim 1, as well as nucleoside analogs in Aim 1, will be expensive and if not commercially available must be custom synthesized off-site for secondary confirmation. Cytotoxicity kits (e.g., CellTiter-Glo) as well as the 96-well plates and other plasticware will be used to examine the toxicity of compounds identified in Aim 1.

PUBLICATIONS ($2,000). Sufficient for two 2 publications per year from Aims 1 and 3.

TRAVEL ($3,000). The budgeted amount will allow travel for 3 investigators to attend one meeting a year for presentation of scientific results and studies, such as American Society for Virology, the International Symposium on Plus-strand RNA Viruses and the International Nidovirus Symposium. These funds will also support two trips per year to UNC for direct meetings (low cost travel and lodging).

A. COMPONENT COVER PAGE

Project Title: Project 3.1 Novel Therapeutic Strategies Targeting Re-emerging Alphaviruses

Component Project Lead Information:

STREBLOW, DANIEL N

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The goal of this project includes identification of novel small molecules capable of inhibiting replication of diverse members of the Alphavirus genus. Alphaviruses are arthropod-transmitted RNA viruses comprising seven antigenic complexes that include multiple Biodefense Category B and C priority pathogens. Alphaviruses are broadly comprised of geographically derived clades: New World [e.g. Eastern (EEEV), Venezuelan (VEEV), and Western Equine Encephalitis (WEEV) viruses) and Old World [e g. Chikungunya (CHIKV), Ross River (RRV), Semliki Forest (SFV), and Sindbis (SINV) viruses]. Two distinct pathologies are manifest during Alphavirus infection. Neurological disease including encephalitis is primarily associated with New World species and can present high mortality rates especially in hosts with weakened or immature immune systems as well as the young and aged populations. Arthralgia and inflammatory syndromes are typically associated with Old World species and while these are uncommonly fatal they can elicit incapacitating effects that persist long after viral clearance. Importantly, CHIKV is currently undergoing a severe re-emergence in areas around the Indian Ocean and Caribbean, an event that has involved evolutionary adaptation allowing inter-host transmission via mosquito species present in North America Currently no FDA approved vaccines or antiviral therapeutics are available to prevent Alphavirus infection or treat Alphavirus-associated disease Importantly, Alphavirus genomes mutate rapidly, greatly facilitating spontaneous changes in their host and vector ranges and virulence, and escape from prior immunity. We have found that the nucleoside analog Ribavirin inhibits CHIKV vRNA synthesis and replication, demonstrating that nucleoside and nucleotide analogs may represent viable therapeutic agents against Alphavirus disease Since the target of this class of inhibitors, namely RNA- dependent RNA polymerase (RnRp) activity, is well conserved among the Alphaviruses, compounds that impact these enzymes should target multiple species and perhaps other RNA virus clades such as Flaviviruses (Project 3), Coronaviruses (Project 2), and Influenza (Project 4) In light of this, experiments outlined in our proposal will utilize an established Alphavirus screening platform to examine a large, previously unexplored chemical library, heavily occupied by nucleoside and nucleotide analogs, by evaluating in vitro replication of two clinically relevant human Alphaviruses namely CHIKV (Old World) and VEEV (New World). This assay has been used to screen a compound library against VEEV and identified >100 that are active against VEEV Subsequent work will involve validation and mechanistic characterization of these efficacious compounds as well as additional ones identified in our primary HTS using unique molecular libraries Our goal is the identification of lead molecules for further in vivo evaluation using both murine and nonhuman primate models of infection. Parallel screening against multiple virus families using the same libraries by other members of this program will dramatically increase the likelihood of identifying antiviral compounds that are efficacious against a broad spectrum of agents In order to develop drug candidates that exhibit antiviral activity against multiple members of the Alphavirus genus we propose the following specific aims:

Aim 1: Employ a validated HTS primary assay to screen novel drug libraries for antiviral compounds that specifically block Alphavirus replication.
Rationale: Southern Research (SR) has developed and validated cell-based, high throughput assays for inhibitors of VEEV and CHIKV induced cytotoxicity Initial use of this assay has already identified several compounds with antiviral activity against VEEV Therefore, these assays will be employed to screen novel libraries of drugs that have not previously been screened against human pathogens including Alphaviruses.
Strategy: A CPE based assay will be used as a primary screen for antiviral compounds with activity against the Alphaviruses VEEV and CHIKV. Following these initial screens, "hits" will be evaluated in dose response and cytotoxicity assays to determine compound-specific EC50 , CC50 , and selective indices.

Aim 2: Validate and characterize antiviral activity and off-target effects.
Rationale: Hit compounds identified in the primary screen could potentially affect any stage of virus replication; therefore, we will characterize the anti-Alphavirus compounds with regard to efficacy and mechanism of action.
Strategy: We will use a variety of secondary assays to identify 1) breadth of anti-Alphavirus activity (test multiple Alphavirus species); 2) cell type-specificity (biologically relevant cells), 3) targets of antiviral compounds, and 4) ease of developing resistance phenotypes. Priority will be given to hits that are efficacious against many Alphaviruses and in multiple cell types, and do not affect virus entry or egress, nor activate IFN.

Aim 3:Chemical optimization and determination of in vivo efficacy of lead compounds
Rationale Our secondary assay characterization is expected to identify multiple compounds that specifically inhibit replication of diverse members of the Alphavirus genus Chemical optimization of effective scaffolds should generate compounds with greater efficacy, selectivity and bioavailability.
Strategy: Hit compounds identified and characterized above will be triaged by the Medicinal Chemistry and Lead Development Core. Compounds with appropriate activity and pharmacokinetic properties will be evaluated using CHIKV and VEEV models of acute and persistent infection and disease

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded Project 3 B2.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Plans For Next Year:

1. Cherry-pick hits. SR will cherry-pick the hits from new CHIKV and VEEV HTS screens using CPE based assays at SR in order to identify compounds with good activity against Alphaviruses. A hits priority list will be established that is based upon IC50/CC50 data and chemical/structural properties of the compounds.

2 CHIKV-mKate(Green). SR has determined that mKate (far red) is not compatible with Mirrorball-HTS Therefore (b)(6) (b)(3)7 ,S.C. § 8401 group is constructing a CHIKV strain expressing Green-mKate, which will be compatible with Mirrorball-HTS at SR for the development of a high throughput screen for the validation of new hits and SAR of analogs.

3 Validate/Mechanism of Action for priority hits at OHSU/UNC/CU. Secondary screens will be used to determine the activity of validated hits Initially, virus reduction assays will be performed Validated compounds will be assessed for the mode of action and the group will attempt to derive resistance mutations against these compounds The Project leaders have established a comprehensive integrated stepwise plan for this part in order to maximize effort and efficiency to improve lead-time for identifying compounds to move forward into SAR.

4 SAR on validated priority hits Active compounds will undergo SAR to identify optimal analogs with increased antiviral activity, solubility and stability as well as decreased cytoxicity This will be an iterative process involving both SR and the Project team.

5. Currently, the group is performing assays to identify the mode of action of the 2 lead compounds that were active against CHIKV and VEEV.
a Tetralins: Additional analogs of SR-34963 (the most active analog of SR-33366) will be synthesized for continued SAR to improve activity Resistance mutations will be identified by RNAseq and the specific mutations will be re-introduced into a sensitive strain in order to identify which mutations confer resistance to this class of drugs. The mode of action of this compound series is being determined utilizing the assays described in SA2. The group will also determine the breadth of activity against additional Alphavirus strains as well as perform activity assays in additional cell types. We expect to identify the mode of action for the Tetralins against CHIKV as well as to identify the most optimal compound in these series over the course of this next year.
b.Quinolones: Additional analogs of SR-34329 (the most active against VEEV) will be synthesized in order to determine whether the thio-amide moeity can be replaced with different R-groups to increase solubility and microsome stability. The first compounds that are proposed to be synthesized will cyclize the thio-amide into a 5-membered ring. Assays are currently under way to identify resistance phenotypes against SR-34329. We are also determining the mechanism of action and antiviral breadth against multiple Alphaviruses in different cell types.

Progress towards our goals is outlined for each Specific Aim:

SA1. HTS Screen of Novel Drug Libraries for Antiviral Compounds that Block Alphavirus Replication

1. 2015 Primary Screening Results: *VEEV HTS* tested 197,025 compounds against VEEV using a CPE based assay in Vero E6 cells (ATCC) at a single concentration. A total of 940 active samples were identified with a threshold percent inhibition greater than 12.12% (>3xSD>mean). *CHIKV HTS* also tested 197,025 compounds against CHIKV using a CPE based assay in Vero E6 cells (Baric Lab) at a single concentration. A total of 2,558 active samples were identified with a threshold percent inhibition greater than 50.38% (>3xSD>mean).
2. Currently, Southern Research (SR) is performing "Cherry-Pick" assays to validate the HTS results for CHIKV and VEEV. Hits will be confirmed and validated by SR in 10-point dose-response assays using the CPE test. These assays are anticipated to be completed by late December 2015. Active hits will be sent to the Project leaders for further validation prior to initializing SAR.
3. SR screened 347,000 compounds against $VEEV_{TC83}$ using Vero cells and 105 hits were identified with an $EC_{50} \leq 10\mu M$ against VEEV-induced CPE. Thirty-five of these compounds were tested using a CHIKV replication assay at OHSU and 4 of them were active (SR-66, -77, -92, and -94). SR derived analogs of two compounds (Tetralin-SR-33366 and Quinolone-SR-33394), which have been used for SAR studies and in mode of action studies.
4. In order to both exclude compounds that block virus replication via activation of type I IFN responses and to enhance virus replication Dr. DeFilippis constructed telomerized human foreskin fibroblast cells that lack IRF3 (THF-ΔIRF3). THF-ΔIRF3 cells allow more rapid and voluminous replication of CHIKV and EEV with more severe CPE. Drs. Streblow and DeFilippis at OHSU have validated four anti-VEEV compounds as effective against CHIKV in these cells. These and other related cell lines were made available to SR and the other Projects.
5. Construction and Sequencing of New CHIKV and VEEV Strains: The Alphavirus group has constructed new strains that will facilitate the next HTS and subsequent validation steps. $CHIKV_{99659}$ was recently isolated from the British Virgin Islands in December of 2013. A low-passage isolate was sequenced and Dr. Heise's group constructed an infectious clone of the virus. This highly relevant strain may not be used for the HTS but it will be used in subsequent validation experiments.

SA2. Validate and characterize antiviral activity and off-target effects

1. The group has developed multiple assays for secondary validation screens that are ready for use against hits identified in SA1. These include CPE-based assays, indirect immunofluorescence assays, and assays utilizing Fluorescent and Nano-Luc-encoding virus constructs.
2. The group has developed assays to identify the mode of action for the lead hits. These will allow us to quickly identify the virus replication step that is inhibited by the compound (e.g. entry, vRNA transcription, translation/processing, virion egress, resistance phenotyping). To prevent duplication of effort and maximize experimental efficiency, each individual laboratory of the Alphavirus group has undertaken the optimization of specific assays that they will utilize to test lead compounds.
3. **Quinolones (SR-33394):** SR synthesized 26 analogs of the quinolone compound SR-33394. OHSU tested them in virus reduction assays and found that SR-33394 had an EC_{90}=0.77μM and only one analog SR-34329 showing improved activity against VEEV in NHDF with an EC_{90}=0.12μM. All other analogs had decreased activity compared to SR-33394. In general, Quinolones were 10-fold less active against CHIKV.
4. **Tetralins (SR-33366):** SR synthesized 35 analogs of the tetralin compound SR-33366 for SAR. OHSU tested them in virus reduction assays with both CHIKV and VEEV. It was determined that in general these compounds were more active against CHIKV. One analog SR-34963 was found to have about a 10-fold increase in activity against CHIKV with an EC90=0.45μM compared to SR-33366 (EC90=3.2μM). The only difference between these two compounds is the presence of a 7-member ring vs. 6-member in the parent compound, which also increased solubility while maintaining microsome stability. OHSU derived a resistance mutant for CHIKV passaged in the presence of SR-34963 and UNC derived a resistance mutant against SR-33366. These drug-resistant viruses are currently being sequenced to identify the mutations associated with resistance.

SA3. Chemical optimization and determination of *in vivo* efficacy of lead compounds

The group has developed a number of models to test in vivo efficacy of lead compounds. These include models of: 1) Acute CHIKV infection and joint disease, including models of disseminated inflammation; 2) Intranasal inoculation of VEEV for neurological infections; 3) chronic CHIKV infection and joint disease in wild-type and immunodeficient mice; 4) Lethal CHIKV and VEEV mouse models; and 5) CHIKV infection of nonhuman primates. Each lab has a unique model that they will be responsible for testing lead compounds that will increase our ability to quickly determine the *in vivo* efficacy profile for each lead.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

File uploaded: C.5.a Other Products-CETR 2015 Streblow.pdf

C.5.b Resource sharing

NOTHING TO REPORT

C.5.a Other Products

Reagents:

1. **THF-ΔIRF-3:** Human foreskin fibroblasts telomerized with pBABE lentivector from AddGene. These cells constitutively express the reverse Tet-transactivator via lentivector (Clontech # 631069); not relevant for this study but just FYI. The IRF3 gene sequence has been disrupted using the CRISPR/Cas9 system (AddGene vector # 49535). The CRISPR lentivector confers resistance to puromycin, which should always be maintained in the culture media @ 3ug/mL (Invivogen Cat # ant-pr-1). The cells are frozen down at 1.8 x 10^6 per vial and can be brought up directly into a T75 + 14mL media. Once confluent they can be split 1:10 for expansion or maintenance. Culture media is 1x DMEM (Fisher Cat#MT-10-017-CV) with 1x pen/strep and 10% FCS (we've used many vendors, e.g. Life Technologies). Cell line constructed by Dr. DeFilippis.
2. **THF-ΔIFIT1, THF-ΔIFIT2, THF-ΔSTING, THF-ΔIPS1, THF-ΔSTAT1:** Human foreskin fibroblasts telomerized with pBABE lentivector from AddGene. These are also stably transduced with a firefly luciferase-coding region under the control of the interferon responsive element using a lentivector obtained from System Biosciences. Individual cell lines were constructed in which the protein coding regions for IFIT1, IFIT2, STING, IPS1, or STAT1 were disrupted using the CRISPR/Cas9 system (AddGene vector # 52961). The CRISPR lentivector confers resistance to puromycin, which should always be maintained in the culture media @ 3ug/mL (Invivogen Cat # ant-pr-1). The cells are frozen down at 1.8 x 10^6 per vial and can be brought up directly into a T75 + 14mL media. Once confluent they can be split 1:10 for expansion or maintenance. Culture media is 1x DMEM (Fisher Cat#MT-10-017-CV) with 1x pen/strep and 10% FCS (we've used many vendors, e.g. Life Technologies). Cell lines constructed by Dr. DeFilippis.
3. **CHIKV Caribbean Strain Infectious Clone:** $CHIKV_{99659}$ was recently isolated from the British Virgin Islands in December of 2013. A low-passage stock of this strain was provided to the members of the Alphavirus group from Dr. Michael Diamond (Project 2). The Heise lab, in collaboration with Dr. Nathanial Moorman at UNC, has sequenced the isolate and constructed an infectious clone of the virus.
4. **$CHIKV_{181/25}$ Strains Expressing nano-Luciferase (nLuc):** Into the infectious clone of $CHIKV_{181/25}$ was introduced an in-frame nLuc reporter gene. Two different viruses were constructed by the Heise Lab: pTH1.2 (NSP-3nLuc) and pTH2.1 (Capsid-nLuc), which will be utilized by SR for cherry-pick validation screens and for mechanism of action studies.
5. **$CHIKV_{AF15561}$ strain expressing mKate:** An in-frame mKate reporter gene was cloned into the infectious clone of the pathogenic parental virus of $CHIKV_{181/25}$ ($CHIKV_{AF15561}$). Constructed by Dr. Morrison's group.
6. **G10:** A novel small molecule (4-(2-chloro-6-fluorobenzyl)-N-(furan-2-ylmethyl)-3-oxo-3,4-dihydro-2H-benzo[b][1,4]thiazine-6-carboxamide) capable of blocking Alphavirus replication by activating STING-dependent activity in human cells was characterized and described by Dr. DeFilippis.

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Oregon Health & Science University

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Daniel		Streblow	PhD	Project Lead	(b)(4) (b)(6)				12,690.00	4,062.00	16,752.00
2.	Victor		DeFilippis	PhD	Co-investigator					11,059.00	3,761.00	14,820.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **31,572.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates	(b)(4)					
1	Graduate Students				28,500 00	0 00	28,500.00
	Undergraduate Students						
	Secretarial/Clerical						
3	Sr. Research Assistant,Research Associate, Microsurgeon				50,601.00	17,775.00	68,376.00
4	**Total Number Other Personnel**					**Total Other Personnel**	**96,876.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**128,448.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0969975150000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: Oregon Health & Science University

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		10,000 00
2. Stipends		0.00
3. Travel		0 00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**10,000.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0969975150000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: Oregon Health & Science University

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

F. Other Direct Costs	Funds Requested ($)*
1 Materials and Supplies	117,200.00
2 Publication Costs	0 00
3 Consultant Services	0.00
4. ADP/Computer Services	0.00
5 Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Animal Costs: Mice Purchase, Sample Collection/surgical supplies, mice perdiem, veterinary time	26,000.00
9. Other Expense.flow Cytometry, slide prep, OTRADI	12,130.00
10. Equipment Maintenance	3,177.00
Total Other Direct Costs	**158,507.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**299,955.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified Total Direct Costs	75.0	289,955.00	217,466.00
		Total Indirect Costs	**217,466.00**

Cognizant Federal Agency DHHS, Arif M. Karim, 415-437-7820
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**517,421.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: Updated Final Budget_Justification_Yr3_WhitleyU19_OHSU_Streblow_Proj3B Dec 2015.pdf
(Only attach one file.)

RESEARCH & RELATED Budget (F-K) (Funds Requested)

Program Director/Principal Investigator (Last, First, Middle): Whitley, Richard J. (Project 3B – Streblow, Daniel N.)

BUDGET JUSTIFICATION, YEAR 3

Streblow/DeFilippis (Project 3B)

PERSONNEL:

Daniel Streblow, Ph.D., Co-Investigator, years 1-5: (b)(4) months (b)(4). Dr. Streblow will serve as the OHSU Subcontract PI of Project 3. Dr. Streblow has extensive experience with animal models of infectious disease and has recently teamed up with Dr. Axthelm to develop a non-human primate model of Chikungunya virus infection and disease. His duties will include maintaining active protocols and animal records, coordinating the animal requirements for this Project, facilitating animal experiments, analyzing samples as well as assembling and disseminating data sets produced during this Project. He will ensure timely completion of the proposed work.

Victor DeFilippis, Ph.D., Co-Investigator, years 1-5: (b)(4) months (b)(4). Dr. DeFilippis will be responsible for *in vitro* experimentation, communicating scientific progress, supervision and training of junior staff, overall experimental design and coordination of drug treatment experiments and determination of mode of action of the antiviral compounds. He is also responsible for data analysis, preparation of reports and publications derived from this part of the Project, as well as communication of research results to the scientific community.

Michael Axthelm, Ph.D., Co-Investigator, years 4-5: (b)(4) months (b)(4). Dr. Axthelm is a veterinary pathologist and an infectious disease specialist with over 20 years of experience investigating mechanisms of viral pathogenesis in non-human primate models, primarily chronic lentivirus and herpesvirus infections. He heads the Infectious Disease Resource that manages the Oregon National Primate Research Center's non-human primate infectious disease protocols. He will advise Al Legasse with respect to coordinating animal selection, protocol implementation, phasing of animal cohorts into the study, and sample and clinical data acquisition. Dr. Axthelm will also advise Mr. Turner in technical aspects of the Project when necessary, including animal sampling procedures, health assessment and anatomic pathology.

Craig Kreklywich, Research Associate, year 3: (b)(4) months (b)(4). He will be responsible for performing quantitative RT-PCR detection of CHIKV in plasma and tissue samples. He will aid the vet team during necropsy. He is involved in immunohistochemical analysis of CHIKV in tissue samples.

Takeshi Ando, M.D., Microsurgeon, year 3: (b)(4) months (b)(4). Dr. Ando is a microsurgeon who has been trained in BSL-2 and BSL-3 virological, molecular biological, and animal work. Dr. Ando will be responsible for assisting Dr. Streblow and will be the primary scientist involved in coordinating, conducting, and processing all *in vivo* experiments involving CHIKV.

Michael Denton, Senior Research Assistant, year 3: (b)(4) months, (b)(4). He will be responsible for producing CHIKV titered stocks for infection studies, processing of animal samples, performing titration, CHIKV detection and flow cytometric experiments.

Alfred Legasse, SPF Project Manager, years 4-5: (b)(4) months (b)(4). Mr. Legasse is the Infectious Disease Resource project manager and has over 25 years of experience working with rhesus macaques as a technician, supervisor and project manager. He will be responsible for scheduling and coordinating day-to-day project activities with the Division of Comparative Medicine animal care staff. He will provide quality assurance for staff training and adherence to standard operating procedures developed to maintain personnel and animal safety and the integrity of the study.

Lisi Amsler, Graduate Student, year 3: (b)(4) months (b)(4) Ms. Amsler will be trained in BSL-2 and BSL-3 virological, molecular biological, and animal work. She will be responsible for assisting Dr. DeFilippis and Dr. Ando in helping to process the experiments involving CHIKV and VEEV.

SUPPLIES (Non-Animal Laboratory)

Antibodies ($2,000/year, years 1-2, $5,000/year, years 3-5)
These are necessary for: 1) Detection of viral replication *in vitro* and for immunohistochemistry; 2) Intracellular cytokine staining assays; 3) Flow cytometry for phenotypic analysis of immune responses to viral infection; and 4) Validation of mode of action studies.

Plasticware/Virus Detection ($47,000/year, years 1-3, $12,000/year, years 4-5)
Disposable plasticware will be required for cell and virus culture, CHIKV titration and virus isolation, and molecular biological work. This includes tissue culture dishes of myriad sizes and layouts, flasks, serological pipettes, disposable pipette tips, microfuge and centrifuge tubes, and disposable screw cap tubes of various sizes for sample storage. This also includes virus detection reagents for quantitative PCR (e.g., Taq polymerase, primers, TaqMan probes, 96-well plates).

Tissue Culture Supplies ($37,200/year, years 1-3, $13,000/year, years 4-5)
These will be required for all cell growth and maintenance as well as virus growth and titration and isolation from tissues. This includes cell culture growth media, animal serum, PBS, trypsin, sucrose, sorbitol, disposable sterilizing filters, antibiotics, and syringes.

Virus Detection Supplies ($20,000/year, years 1-3, $13,000/year, years 4-5)
qRT-PCR will be used for the detection of both CHIKV and DENV. Reagents for virus detection include: Reverse transcription reagents, ABI Master mix containing Taq polymerase, virus-specific primers and TaqMan probes, 96-well optical plates.

HTS reagents ($5,000/year, years 1-3)
These will be required for the detection and quantification of CHIKV and VEEV replication during the HTS assays and include reagents for luciferase detection and CellTiter-Glo Luminescent Cell viability assays. Special 96-well optical plates will be required for these assays.

LN_2 and CO_2 ($3,000/year, years 3-5)
Liquid nitrogen and carbon dioxide will be needed for incubators, cryopreservation, enzymes for tissue digestion, and tissue fixatives.

SUPPLIES (Animal):

Mice purchase ($10,000/year, years 1-2, $12,000 in year 3)
We will purchase approximately 400 C57/Bl6 mice (3-4 weeks of age) from Jackson Laboratories ($16.40/animal)= $6,560. We will also determine the effect of the antiviral drug on virus replication and disease in a more stringent mouse model (e.g., mice lacking the IFN-dependent effector STAT1 (STAT1). We will maintain a colony of STAT-/- mice. The number of breeding cages and the cages required to maintain weaned mice to support the proposed experiments will be calculated according to guidelines proposed in "Breeding strategies for Maintaining Colonies of Laboratory Mice" published in 2007. Approximately 20 breeder mice will be purchased from Taconic Farms, Inc. (www.taconic.com) ($160.50 each=$3,210). We will maintain approximately 15 breeder pairs at all times in years 1-3. Per diem cage costs for these mice should total $10,000 each year for Years 1-3 (assuming a total of 1 month housing at $1/cage/day for experimental mice and 365 days/cage/year for breeders).

Rhesus macaque lease fees ($51,529/year, years 4-5 only)
Rhesus macaques cost $6,440/animal between the ages of 5-11 years old. The lease fees reflect the portion of the true production costs, and are standardized for all Public Health Service grantees using the ONPRC.
Years 4-5: 8 animals x $6,440 = $51,520 per year.

Rhesus macaque set-up fees ($1,472/year, years 4-5 only)

$184/animal, are charged by the Division of Comparative Medicine to defray the administrative costs of animal selection, records requirements for assignment and initial health assessment to insure healthy animals are assigned to projects. Years 4-5: 8 animals x $184 = $1,472 per year. LN2 and C02 ($3,000/years 3-5)
To support these studies, we will require LN2 and C02.

Sample collection and drug and agent administration (Surgical Supplies) ($1,000/year, years 1-3; $2,000/year, years 4-5)
Vacutainer blood tubes, needles, syringes and sterile plastic collection tubes and swabs required for obtaining blood samples and tissues from both NHP and mice.

DomesticTravel ($3,000/year, years 1-5)
$3,000/year for Co-Investigators to attend an international meeting pertaining to antiviral therapeutics directed against emerging RNA viruses.

OTHER EXPENSES:

Largely fees assessed for animal maintenance (per diem), and fees for surgical services provided by the ONPRC Division of Comparative Medicine staff. The experiments described for years 1-3 were designed to test larger numbers of compounds (or refined compounds) in a mouse model of CHIKV infection and disease. However, in years 4-5 we will test 1-2 of the best candidate compounds in a Rhesus macaque model of CHIKV infection and disease. The prices per year reflect the experimental design.

The number and cost of each of these items are provided in the following other expenses summary:

Rhesus macaque per diem, ABSL 3 ($5,808/year, years 4-5 only)
$51.86/animal/day for 14 days for 8 monkeys per year in FY4&5.

Necropsy fees ($16,376/year, years 4-5 only)
$2,047/animal, 8 animals/year for Years 4 and 5 only (Grade 3 gross necropsy + histopathology).

Estimated number of NHP: Years 4-5, 8 animals.

Mice per diem ($10,000/year, years 1-3, only)
We will purchase approximately 400 C57/Bl6 mice (3-4 weeks of age) from Jackson Laboratories ($16.40/animal)=$6,560. We will also determine the effect of the antiviral drug on virus replication and disease in a more stringent mouse model (e.g., mice lacking the IFN-dependent effector STAT1 (STAT1$^{-/-}$). We will maintain a colony of STAT-/- mice. The number of breeding cages and the cages required to maintain weaned mice to support the proposed experiments will be calculated according to guidelines proposed in "Breeding strategies for Maintaining Colonies of Laboratory Mice" published in 2007. Approximately 20 breeder mice will be purchased from Taconic Farms, Inc. (www.taconic.com) ($160.50 each=$3,210). We will maintain approximately 15 breeder pairs at all times in years 1-3. Per diem cage costs for these mice should total $10,000 each year for Years 1-3 (assuming a total of 1 month housing at $1/cage/day for experimental mice and 365 days/cage/year for breeders).

Flow cytometry charges ($3,000/year, years 1-5)
We are charged $60/hour of FCM time, which will be used to analyze peripheral blood samples for specific cellular markers as well as when performing intracellular cytokine staining assays from both mice and NHP. We are estimating 50 hours of FCM time per year.

Veterinary time (physical exams) ($3,000/year, years 1-5)
$30.26/animal/hour. We expect roughly 100h total vet time/ yr. Includes analysis of unexpected complications arising from dugs/infections in mice and NHP.

Slide Preparation and Histology ($4,000/year, years 1-5)
We are charged $4.48/block for processing of tissue samples, $1.39/section for cutting and mounting, and $0.77/slide for H&E staining. We are estimating that we will need $4,000/year for slide preparation and staining.

OTRADI services ($5,130/year, years 1-3 only)
The proposed library screen and validation will be conducted at the VGTI and the plates will be read by the automated fluorescence microscope at Oregon Translational Research and Drug Development Institute (OTRADI). Cost is $50 for setup and $125/hour + 35% overhead. We estimate 10 plates/library in triplicate (=30) for 3 libraries (=90 plates). Each plate takes approximately 20 minutes to read (1,800 minutes=30 hours total time) = [$50 + (30 x $125) = $3,800 + 35% ($1,330) = $5,130]. We expect screens of CHIKV and VEEV to be conducted over Years 1-3.

Equipment Maintenance ($3,177/year, years 3-5)
This proposal will require the use of our general laboratory equipment, which must be maintained to properly execute this study. Therefore, we are requesting $2,235 in years 3-5 to maintain the equipment in good working order.

A. COMPONENT COVER PAGE

Project Title: Project 4.1 Identification and characterization of novel drugs that target the Influenza virus polymerase functions

Component Project Lead Information:

Whitley, Richard J.

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The overall goal of this project is to identify new therapies that target influenza virus replication The emergence of highly pathogenic strains of influenza virus has highlighted the urgent need for new effective treatments A primary concern with the current drugs used to treat influenza is the development of resistance mutations that negate therapeutic benefit. Antiviral resistance to the M2 ion channel inhibitors (adamantanes) increased sharply in Asia beginning in 2002 Subsequently, by 2005 in the United States, 92% of influenza A (H3N2) isolates had developed high level resistance to this class of drugs. Currently, the CDC recommends that neither amantadine nor rimantadine be used for the treatment or chemoprophylaxis of influenza A in the US. Evidence in the literature suggests that targeting the influenza virus RNA dependent RNA polymerase (RdRp) is a rational approach for antiviral therapy The RdRp is responsible for a number of functions including 5'cap recognition, endonuclease activity, replication, transcription, and polyadenylation. Recently, cryo-EM reconstitution studies identified branched-RNP structures as putative replication intermediates and suggested a mechanism for viral replication by a second polymerase activity on the RNP template {Moeller, 2012 #383}. The second polymerase activity is believed to be a function of the polymerase complex Clearly, the RdRp provides multiple functional domains that could be targets for antiviral drug therapy Previous studies showed that mutations in the conserved regions of PB1 subunit of the polymerase complex produce inactive RNA polymerase {Biswas, 1994 #389}. We hypothesize that the viral escape mutants resulting from drugs targeting the influenza polymerase might produce inactive RdRp that is unable to replicate the viral genome

The specific aims of the original proposal

Aim#1. Employ a validated HTS primary assay to screen novel drug libraries for antiviral compounds that specifically block influenza virus replication.

Hypothesis and rationale Targeting the influenza polymerase activity might prove more effective than targeting the viral glycoproteins. There are multiple functional domains of influenza polymerase that are rational targets for antiviral therapy Previous studies demonstrated that point mutations of the conserved regions of PB1 produce inactive polymerase. We hypothesize that viral resistance to novel compounds that target the polymerase activity might attenuate or inactivate the viral polymerase It is also likely that we will identify compounds against the conserved regions of influenza virus polymerase subunits that might be effective against multiple viral strains. Experimental strategy We will use established CPE-based assays to screen novel libraries against influenza viruses We will use this assay to screen small molecule libraries that have not been previously screened for activity against human pathogens. These libraries are composed of highly diversified small molecules that contain novel and original drug-like features with distinct topologies and diverse functionalities.

Aim#2: Characterize the antiviral activity of hit compounds and identify anti-polymerase inhibitors

Hypothesis and rationale: The CPE-based HTS screening will identify hit compounds that target several stages of the virus life cycle, including multiple functional domains of the influenza RNA polymerase. It is critical to design an experimental strategy that will focus our analysis on the hit compounds that block post-entry steps of viral infection.
Experimental strategy: To identify the hit compounds that target the viral polymerase we will first use a viral entry assay to eliminate hit compounds that target the first step of the infection cycle Elimination of hit compounds that target the interaction of the virus glycoprotein with the host cell receptor will focus the search for post-entry inhibitors including anti-polymerase. Additionally, we will eliminate hit compounds that induce interferon. Several secondary and tertiary assays will be performed to determine the viral protein target of the hit compounds.

Aim#3: Chemical optimization and determination of the in vivo efficacy of lead compounds

Hypothesis and Rationale: Our secondary assay characterization is expected to identify multiple compounds that are specifically effective in inhibiting influenza replication. Optimization of the effective scaffolds should generate compounds with greater efficacy, selectivity, and bioavailability.
Experimental strategy The hit compounds from the HTS will be triaged and progressed as outlined in the Chemistry core Compounds with the appropriate activity and pharmacokinetic properties will be evaluated using in-house mouse infection models

There are no changes in either the goals or specific aims as originally submitted.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Project 4 B.2 done 12.7.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Plans for year 3 - March 1, 2016 – February 28, 2017

Once an acceptable cell substrate for an HTS assay is identified, the HTS conditions will be optimized and the assay will be assessed for robustness and reproducibility. A larger set of compounds was obtained from Gilead Sciences and will be used in a small pilot study to confirm that the assay is performing as expected The assay will also be adapted to conditions required by the Southern Research HTS facility. When hits from the HTS facility are identified, cherry picked compounds will be rapidly evaluated to help identify promising series of compounds and support hit to lead optimization by the medicinal chemistry core. Reverse genetic systems are also in place and will be used to identify the precise molecular targets from lead compounds identified in Project 4.

B2.1. The overarching goal of Project 4 is to identify inhibitors of the influenza virus (RdRP). Currently, there are only two direct acting antiviral drugs that target this enzyme complex that have been approved or are in advanced stages of development, T-705 and VX-787. While the RdRP is clearly a high priority target, biological limitations of influenza virus replication in cell culture systems have impeded efforts to identify molecules that target this complex. A major aim of this project is to develop a primary cell-based assay that can overcome technical limitations of influenza replication, specifically the use of cytopathology as an endpoint and the use of a canine cell substrate (MDCK cells). To accomplish this, a Nanoluc expressing isolate of A/California/7/2009 was obtained for use in a primary infectious assay that eliminates the need for cytopathology as an endpoint (1). This approach affords new opportunities in the choice of cell lines for use in a primary assay.

An efficient 384-well assay was developed that yields accurate EC_{50} and CC_{50} for several known RdRP inhibitors and provides proof of principle for this experimental approach. A large stock of a plaque purified virus isolate was then produced in preparation for an HTS screen. While this initial assay was performed in MDCK cells, the general experimental approach is being applied to a set of additional human cell lines to identify candidate cell lines for a primary assay. The choice of a human cell line is important as it should more accurately reflect the anabolism of nucleoside analogs that are likely to inhibit the RdRP complex. Each cell line was evaluated for i) tolerance of serum free conditions, ii) stability to trypsin and BSA in culture media, iii) support of influenza virus RNA replication, and iv) expression of Nanoluc activity. Human cells evaluated thus far include A549, HFF, HEL299, HEK 293, 16-HBE, and primary normal human bronchial epithelia. While none have proven to support sufficient levels of virus replication, primary normal human bronchial epithelial cells and primary small airway epithelial cells are being evaluated. Additional cell lines are being obtained and evaluated on a continuing basis until a suitable cell line is identified.

An efficient yield reduction assay was also developed using CellTiter-Glo as an endpoint to provide more precise information on the activity of compounds identified in the primary assay. The throughput is sufficient to support medicinal chemistry efforts and will also be used with currently circulating H1N1, H3N3, and B strains of influenza virus to provide spectrum of antiviral activity data.

B2.2. Identification of compounds active against Influenza A virus pandemic (H1N1) and seasonal (H3N2) subtype. During the previous year, in a single dose pilot study, the HTS core identified 56 compounds that were active against the Influenza A virus (IAV) H3N2 subtype at a concentration of ≤30 μM. In the follow up investigations, we analyzed these compounds against pandemic (A/California/7/2009, H1N1) and seasonal (A/Udorn/72, H3N2) subtypes in dose response studies. This was done using the human HEK293 Flu-Luc reporter cell line (2). Each compound was tested at various concentrations ranging from 50 – 0.26 μM. The results indicated that of the 56 compounds, 24 had inhibitory activity against both H1N1 and H3N2 subtypes with an IC_{50} value of <20 μM (majority of these compound had IC_{50} of <10 μM for both subtypes). In parallel studies, we evaluated cytotoxicity of these compounds and found that none displayed any significant cytotoxic effects (Selectivity Index >20 for majority of the compounds).

Counter screens – Haemagglutinin and Neurominadase assays. As stated, the goal is to identify compounds that affect the RNA-dependent RNA polymerase. Therefore, it was critical to prioritize the confirmed hit compounds prior to further analysis or initiating chemistry. A series of counter-screens were established to identify the compounds with potential activity against IAV haemagglutinin (HA) or neuraminidase (NA). The assays were established and optimized and the 24 compounds from above were tested. The results indicated that none of the compounds were HAI or NAI inhibitors at any of the concentrations tested (50 – 0.26 μM).

B2.3. Analysis of compounds in IAV RNA-dependent RNA polymerase assay. In order to determine if the compounds inhibited the IAV RdRP, we employed a minigenome RdRP assay (3). The assays was adapted and optimized to a 96-well plate format, and subsequently the compounds were analyzed in a concentration response assay (50 – 0.26 μM). Of the 24 hit compounds, three compounds (SRI34518, SRI35129 and SRI35136) exhibited significant reduction of RdRP activity

with IC_{50} values of approximately 3.0, 12.0 and 3.0 μM, respectively.

B2.4. Optimization of a viral entry assay for IAV. Positive hits coming from the primary HTS assay will be evaluated in cell entry assays to determine if inhibitory drugs are targeting viral cell entry. We produced viral particles pseudotyped with H5N1 or H1N1 IAV envelope proteins with a core consisting in the genome of VSV containing a luciferase reporter gene. When target cells are infected with the pseudotypes, the luciferase is expressed after cell entry occurs and light signal can be quantitated. Signal produced by H5N1 pseudotype was more than two orders of magnitude higher than the null control, while H1N1 signal was at least 5-fold higher than the null control when the entry assay was done in 293FT cells. Experiments are ongoing to improve H1N1 signal. Specificity of the signal was tested by measuring the ability of pseudotypes to hemagglutinate chicken erythrocytes. Only H5N1 and H1N1 particles were able to agglutinate red blood cells, but not the null or VSV pseudotypes. The H5N1 and H1N1 pseudotypes obtained can be used to evaluate the effect of inhibitory drugs on viral cell entry

B2.5. Compound SRI 34518 also inhibits infection of H5N1 subtype. To determine if any of the compounds that inhibited H1N1 and H3N2 subtypes (section B2.1) were also active against the highly pathogenic avian influenza A virus (HPAI) subtype, the HTS core tested the compounds against A/Vietnam/1203/2004, H5N1. MDCK cells were infected with the virus in the presence of various concentrations of each of the compounds and virus-mediated cell death (cytopathic effect, CPE) was measured 72 h post-infection. The results indicated that of the 24 hit compounds tested, only SRI 34518 significantly inhibited the virus-mediated CPE with an IC_{50} of 7.006 μM. Thus, the compound SRI 34518 has broad-spectrum activity against all three influenza subtypes.

Optimization of a RT-qPCR secondary assay for viral quantification of IAV. A RT-qPCR assay for quantification of IAV was developed as a secondary assay to confirm hit compounds that would be identified from the primary HTS assays. The primers and probe used specifically recognize the M2 gene in segment 7 of IAV genome from diverse strains of IAV. To test the functionality of the assay, we tested the RT-qPCR using viral samples taken from cytopathic effect (CPE) reduction assays using ribavirin and VXb as controls. The CPE assays were done using MDCK and A549 cell lines. Cells were challenged with H1N1 (A/Ca/7/2009) and H3N2 (A/Udorn/72) IAV strains. The qRT-PCR was able to detect a reduction in the amount of viral load with increasing concentrations of compounds, which was comparable with the reduction observed in the CPE assay. Using appropriate controls to discard a possible inhibitory effect of the compounds on the reverse transcriptase or the polymerase used for the RT-qPCR, we demonstrated that the RT-qPCR is reliable and can be used as a secondary assay to test IAV inhibition by hits from the primary HTS assay.

Summary. During the year 2, we have made considerable progress towards establishing and optimizing various assays such as Flu-Luc reporter cell line assay for confirmation of the hits, and HAI, NAI and RdRP assays for mechanism of action studies. In addition, in a pilot screen we have identified one broad-spectrum compound that is active against the pandemic (H1N1), seasonal (H3N2), and HPAI (H5N1) IAV subtypes, and 23 compounds that are active against pandemic and seasonal subtypes. Of these 24 hit compounds, 3 are specific inhibitors of IAV RdRp. Currently, we are developing additional assays to decipher the exact mechanism of action of these compounds.

References:

1. Tran V, Moser LA, Poole DS, Mehle A. Highly sensitive real-time in vivo imaging of an influenza reporter virus reveals dynamics of replication and spread. J Virol. 2013;87(24):13321-9.
2. Li, Y., Larrimer, A., Curtiss, T., Kim, J., Jones, A., Baird-Tomlinson, H., Pekosz, A. & Olivo, P. D. (2009). Influenza virus assays based on virus-inducible reporter cell lines. Influenza Other Respir Viruses 3, 241-251.
3. Ortigoza, M. B., Dibben, O., Maamary, J., Martinez-Gil, L., Leyva-Grado, V. H., Abreu, P., Jr., Ayllon, J., Palese, P. & Shaw, M. L. (2012). A novel small molecule inhibitor of influenza A viruses that targets polymerase function and indirectly induces interferon. PLoS Pathog 8, e1002668.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 063690705

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Richard		Whitley	MD	Project Lead	(b)(4), (b)(6)				18,330.00	5,517.00	23,847.00
2.	Mark		Prichard	PhD	Co-investigator					13,881.00	4,178.00	18,059.00
3.	Debra		Quenelle	PhD	Co-investigator					12,255.00	3,689.00	15,944.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **57,850.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
3	Research supervisor, 2 research technicians	(b)(4)			23,839.00	8,272.00	32,111.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**32,111.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**89,961.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 063690705
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0 00
2. Stipends		0.00
3. Travel		0 00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 063690705
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	16,319.00
2. Publication Costs	750.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Sequencing, Animal per diem	4,678.00
9. Repair and Maintenance, shipping	1,250.00
Total Other Direct Costs	**22,997.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**115,958.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	47.0	115,959.00	54,500.00
		Total Indirect Costs	**54,500.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**170,458.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: Budget justification Proj 4.1.pdf
(Only attach one file.)

RESEARCH & RELATED Budget (F-K) (Funds Requested)

Program Director/Principal Investigator (Last, First, Middle): Whitley, Richard J. (Project 4.1)

Budget Justification

Personnel

Richard J. Whitley, MD, PD/PI (b)(4) **months:** Dr. Whitley will continue to serve as the UAB Program Director/Principal Investigator for Project 4 investigating the inhibitors of influenza virus. He continues to provide broad oversight of the project and serves as primary liaison with the External Advisory Board, NIH and other external entities, including pharmaceutical companies to determine potential compounds to be developed, and international groups such as the IDSA to determine therapeutic needs.

Mark Prichard, PhD (b)(4) **months**: Dr. Prichard has conducted research in discovery and development of antiviral drugs for more than twenty years. He continues as the PI of an NIAID contract focused on the evaluation of compounds for antiviral activity against the human herpesviruses and the orthopoxviruses. During this past year, he has expanded his role on this project and in the coming year, he will continue to work closely with Southern Research on development of assays and evaluation of compounds from the HTS work being completed. He will also oversee the testing of drug resistance

Debra Quenelle, DVM, PhD. (b)(4) **months:** Dr. Quenelle has more than 26 years of experience in use of animal models in infectious diseases, and is the PI for an NIAID contract to test compounds in animal models. Preliminary animal work, which is underway at the time of this report, will continue in Year 3. Dr. Quenelle will oversee the in vivo studies in mice to determine the antiviral activity and toxicity of the compounds identified in the compound screening.

Kathy Keith, MS, Laboratory Supervisor (b)(4) **months,:** Ms. Keith more than 24 years of experience in laboratory work on a number of viruses including HIV, α&β-herpes, influenza, vaccinia and cowpox primarily determining in vitro drug efficacy using different endpoint methods (e.g., ELISA, CPE, plaque reduction, virus yield, hybridization, real time PCR) and under Biosafety levels 2 - 3. She will continue to oversee the antiviral assays and day to day activities for this project in Dr. Prichard's laboratory.

Deborah Collins, Research Technician (b)(4) **months,** Ms. Collins has more than 20 years of experience working with experimental animal studies, both large and small animals. She will continue to assist with the mice studies which are beginning for the project

Terri Rice, Research Technician, (b)(4) **months, :** Ms. Rice has 17 years of prior experience with small animal toxicology and pharmacology studies. She will continue to assist with all animal studies performed as part of the project.

Supplies

Funds are requested for tissue culture, reagents, surgical supplies, PPE, labware and miscellaneous laboratory supplies need to conduct the planned compound testing. In addition, in funds are included to provide for purchase of mice to be used in the in vivo testing.

Travel

Funds are requested to allow travel of the PD/PI and co-investigators to attend CETR or related scientific meetings to present results of the study.

Other Expenses

Funds are requested for shipping of materials, sequencing, maintenance of project specific equipment, and publication costs. Costs are also requested to cover per diem costs for care of mice being used in the studies.

A. COMPONENT COVER PAGE

Project Title: Screening Core - Core B

Component Project Lead Information:

(b)(6), (b)(3) 7 U S C § 8401

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

B1: Major goals

The overarching goal of the Screening Core (SC) is to identify chemical series with anti-viral effects in high throughput screens against multiple virus targets and to assist in converting them into drugs by providing in vitro biological screening support to the Medicinal Chemistry and Lead Development Core (MCLDC). By screening a unique, common compound collection against each virus, the screening core seeks to identify selective as well as broad-based inhibitors of viral replication in accordance with the theme of the program.

Specific Aims

Aim 1: Identify hit compounds for influenza, dengue, Venezuelan equine encephalitis, West Nile, Chikungunya viruses, and SARS Coronavirus. The overall aim of the SC is to identify hit compounds that inhibit replication of influenza (INFV), dengue (DENV), Venezuelan equine encephalitis (VEEV), West Nile (WNV), Chikungunya (CHIKV), and/or SARS CoV. A cytoprotection effect (CPE) assay will be employed for screening against VEEV, WNV, CHIKV, DENV and SARS CoV. Different assay readouts will be investigated for screening INFV, including a reporter gene and viral titer assays The CPE assay for SARS CoV will be run in multiple conditions to identify inhibitors of virus replication by unknown mechanisms of action as well as those specifically targeting CoV fidelity and RNA capping Each of the viruses will be screened using the same 300,000 member library that was selected due to its unique properties with regards to chemical diversity, drug-like properties and potential ability to modulate a variety of biological pathways and targets involved with viral replication. By using a common library for all of the assays, compounds that are active across several viruses may be identified and could result in the identification of targets with broad spectrum activity Primarily this will be accomplished by sharing the compounds among the consortium participants and by establishing an Antiviral Drug Discovery and Development Consortium (AD3C) database using Enterprise Content Management Documentum CenterStage, where all of the assay conditions and screening results will be uploaded All the Consortium participants have access to this secured site.

Aim 2: Perform the assay(s) for each virus to be used to provide the biological support for each virus for structure-activity studies by the Medicinal chemistry and Lead Development Core (MCLDC). As the hits from the CPE assays, CoV fidelity and end capping assays and the INFV assays are developed, a moderate throughput assay is needed for each target to quantify the changes in activity that occurs as structural modifications are made to the hit compounds These assays (SAR driving assays) will be used in the design-make-test cycle to determine structure-activity relationships that will be important for developing lead series and compounds suitable for in vivo testing. As additional mechanistic studies are completed by the various groups, supplemental cell based or biochemical assays may be incorporated into the project.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Core B B2 pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

B 6. Plans for next reporting period
Following completion of all HTS campaigns, the SC will conduct anti-viral assays relevant for each project to determine IC50 values for newly synthesized compounds produced by the MCLDC as listed below. This work will be an integral component of the iterative design-make-test cycle during the lead generation phase of these projects (see timeline in Section G; Core Specific information).
Project – Assay - Readout
DENV - viral protein expression - fluorescence

WNV - CPE (host cell viability) - luminescence
SARS - virus reporter (nanoluc) - luminescence
CHIKV - virus reporter (fluorescent protein) -fluorescence
VEEV - CPE (host cell viability) - luminescence
FLUV - virus reporter (nanoluc) - luminescence

B2: Accomplishments under these goals

1. The DENV HTS campaign has been successfully completed. A Dengue viral stock prepared in insect cells was needed to improve CPE assay performance required for HTS. A total of 304,810 compound samples were tested once at a single concentration. Using a threshold % inhibition ≥ 26.25% (>3xSD from no effect mean), 240 samples were identified as active and retested at 10 concentrations for anti-viral CPE and direct cytotoxicity effects in host cells. Of these, 45 compounds were confirmed and validated as hits. Subsequent to the completion of the HTS campaign, an SAR driving assay measuring viral protein expression in the host cell was developed. The assay is currently being used to confirm activity of the HTS hit compounds and to test analog and newly synthesized compounds based on those hits.

2. The SARS HTS campaign has been successfully completed. A total of 305,648 compound samples were tested once at a single concentration in 17 batches. Using a threshold % inhibition ≥ 80%, 2492 samples were identified as active and retested at 10 concentrations for anti-viral CPE and direct cytotoxicity effects in host cells. Of these, 874 compounds were confirmed and validated as hits. Subsequent to the completion of the HTS campaign, an SAR driving assay measuring reporter luminescence as an index of virus titer was developed using the recombinant SARS Nanoluc virus produced in the Baric lab. The assay has been used to test over 300 compounds (HTS hit compounds as well as analog and newly synthesized compounds based on those hits) to support ongoing development of SAR.

3. The primary screening phase of the HTS campaigns for both CHIKV and VEEV has been completed. A total of 197,025 compound samples were tested once at a single concentration in 12 batches in each assay. For CHIKV, 2558 samples were identified as active using a threshold % inhibition ≥ 50.38% (>3xSD from no effect mean). For VEEV, 940 samples were identified as active using a threshold % inhibition ≥ 12.12% (>3xSD from no effect mean). Work is ongoing to retest these compounds at 10 concentrations for anti-viral CPE and direct cytotoxicity effects in host cells to confirm and validate hits.

4 Three different assay methods have been investigated for screening against influenza A virus. The first of these measures a reporter gene activity (luciferase expression) to detected virus infection. A second assay measures expression of a viral protein on the surface of infected host cells. In this method, a fluorophore conjugated antibody bound to a flu virus protein expressed on the surface of an infected host cell is detected using laser scanning cytometry to quantify virus production. Both assays were optimized with performance characteristics suitable for HTS. A collection of 1120 biologically active compounds (Prestwick library) were tested at 4µg/ml on two separate days to determine robustness and reproducibility of the assay when run under HTS conditions. Both assays exhibited good reproducibility with signal to background ratios > 5, CVs < 10% and Z' factors >0.5 but were not able to detect nucleoside compounds with known anti-viral activity against influenza as determined in neuraminidase and CPE assays. Therefore, we have decided to develop an assay using a Nanoluc reporter virus similar to that currently being used to support SAR in the SARS project. The parameters of this assay are currently being optimized for performance in HTS.

5. Efforts to develop a suitable assay for WNV using MEF cells have been problematic due to variability in CPE. After discussion with the project team we have decided to evaluate HEK 293 cells with inducible ectopic expression of IFIT. This cell line has recently been obtained by SC and is being characterized for growth conditions and CPE effect with and without induction of IFIT.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

F 2: Challenges and delays
Due to budget limitations, the screening collection was reduced to 222K compounds (by omitting the Chembridge Set 3) for the HTS campaigns against CHIKV, VEEV, WNV and FLUV.
Attempts to utilize newly created cell lines that had not been characterized for use in HTS resulted in unanticipated expenditure of time, effort and resources to develop suitable assays for each virus target. As a consequence, we anticipate that completion of the HTS campaigns for WNV and FLUV will extend into the beginning of year 3.

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

File uploaded: Core B F3.pdf

F.3: Significant changes to select agents

Following CDC approval, the recombinant SARS CoV (Urbani strain) expressing a NanoLuc reporter was transferred to [redacted] This virus is currently being used in assays to evaluate newly synthesized compounds for development of SAR.

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RPPR - Core-5323

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		(b)(6), (b)(3) 7 U S C § 8401			PhD	Project Lead	(b)(4) (b)(6)				16,304.00	7,304.00	23,608.00
2.					PhD	Co-Project leader					12,793.00	5,731.00	18,524.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **42,132.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
13	5 biologis, 2 Informatics Spec, 1 Scientist, 4 Support Staff, BSL3 pay differential ($2 00x800 hours)	(b)(4)			109,000 00	48,114.00	157,114.00
13	**Total Number Other Personnel**					**Total Other Personnel**	**157,114.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**199,246.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0069005260000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,500.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**2,500.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0 00
2. Stipends		0.00
3. Travel		0 00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0069005260000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

F. Other Direct Costs	Funds Requested ($)*
1 Materials and Supplies	200,418.00
2 Publication Costs	0 00
3 Consultant Services	0.00
4. ADP/Computer Services	0.00
5 Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. HTS Robot hours	99,000.00
9. BSL3 facility charge	53,280.00
Total Other Direct Costs	**352,698.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**554,444.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. OH - Salaries + Benefits	120.0	199,246.00	239,097.00
2. G&A - Total Direct Cost + OH	20.0	793,542.00	158,708.00
3. CFC - Salaries + Benefits	4.6	199,246.00	9,165.00
4. CFC - Total Direct Cost + OH	1.0	793,542 00	795.00
		Total Indirect Costs	**407,765.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**962,209.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Budget Justification Core B.pdf (Only attach one file.)

RESEARCH & RELATED Budget (F-K) (Funds Requested)

Year 3 Budget Justification for the Screening Core

(b)(6), (b)(3) 7 U S C § 8401, Ph.D. will serve as Project Leader of the screening core. (b)(6), (b)(3) 7 U S C § 8401 (b)(6), (b)(3) 7 U S C § 8401 combined experience in academia and the pharmaceutical industry involving work in over 60 drug discovery programs. (b)(6), (b)(3) 7 has expertise in the development and use of biochemical and cell-based assays in HTS with a focus on translational relevance to ensure that HTS output can be effectively exploited as part of a comprehensive approach to chemical probe and lead generation. (b)(6), (b)(3) will have oversight for the automation and execution of the high throughput screens, counter and specificity screens, and biological support for the SAR studies. In collaboration with the PIs and other Co-Investigators, (b)(6) will assist in interpretation of the biological results; report, manuscript and patent preparation; and overall project management. (b)(6), (b)(3) 7 will devote (b)(4) calendar months in YR3 to this program.

(b)(6), (b)(3) 7 U S C § 8401 will serve as Co-Project Leader and has overseen the screening of large compound libraries against enzyme, protein, and cell-based assays for both government and commercial clients as Director of the SR HTS Center from 2004 until 2013. (b)(6), (b)(3) 7 U S C § will assist (b)(6), (b)(3) 7 U S C § 8401 by lending (b)(6), (b)(3) expertise in infectious disease HTS and probe/drug development as well as management of large inter-institutional collaborations. In collaboration with the PIs and other Co-Investigators, (b)(6), (b)(3) 7 will assist in interpretation of the biological results and report, manuscript and patent preparation. (b)(6), (b)(3) 7 will devote (b)(4) R3 to this program.

(b)(6), (b)(3) 7 U S C § 8401 M.S., will supervise and manage the day to day efforts for assay development and screening including scheduling, equipment maintenance and QCing, and data QC. (b)(6), (b)(3) 7 U S C § rings her expertise in keeping the Center with an annual operating budget of over $3,000,000 operating efficiently. (b)(6), (b)(3) 7 U S C will devote (b)(4) months in YR3 to the project

(b)(6), (b)(3) 7 U S C § 8401 MS, PMP has five years experience in coordinating and managing research projects. (b)(6), (b)(3) 7 will work with Dr. Suto and the other project leaders to ensure a timely and efficient delivery of Core services to the overall program and will devote (b)(4) months in YR3 to the program.

HTS Center Personnel – will be responsible for executing the biological assays driving SAR including compound handling and informatics support for data analysis:

(b)(6), (b)(3) 7 U S C § 8401 M.S., will be responsible for compound management and drugging for the biological assays. (YR3 (b)(4) months)

(b)(6), (b)(3) 7 U S C § 8401 B.S., will assist (b)(6), (b)(3) 7 U S C § 8401 in drugging for the HTS. (YR2 (b)(4) months)

(b)(6), (b)(3) 7 U S C § 8401 B.S., oversees the HTS informatics group and will be responsible for writing the data templates for the screening effort and data import and analysis and depositing the data with Enterprise Content Management Documentum CenterStage database. (b)(6), (b)(3) 7 U S C § 8401 manages our ActivityBase software, the Oracle database, and will facilitate transfer of data between the groups including the cheminformatics staff. (YR2 (b)(4) months)

(b)(6), (b)(3) 7 U.S.C. § 8401 will be responsible for importing the data from the plate readers into the analysis software and generating data reports. (YR2 (b)(4) months)

(b)(6), (b)(3) 7 U.S.C. § 8401 M.S. will be responsible for the statistical analysis of the high throughput screening data. (b)(4) months)

(b)(6), (b)(3) 7 U.S.C. § 8401 will be responsible for running the SAR driving anti-viral assays for all virus targets (b)(6), (b)(3) 7 U.S.C. § 8401 has BSL3 certification and is trained to work with select agents. (b)(6), (b)(3) 7 U.S.C. efforts will include assay automation and data verification. (b)(4) months)

(b)(6), (b)(3) 7 U.S.C. § 8401 M.S., will assist in the execution of the SAR driving assays requiring work in the BSL-3 and will be responsible for preparing cells, media, reagents, barcoding plates, and reading plates and the execution of the cell cytotoxicity assays. (b)(4) months)

(b)(6), (b)(3) 7 U.S.C. § 8401 B.S., will assist in the execution of the SAR driving assays in the BSL-2 containment lab and will preparing cells, media, reagents, barcoding plates, and reading plates and will be responsible for execution of the cell cytotoxicity assays. (3.6 cal months)

(b)(6), (b)(3) 7 U.S.C. § 8401 B.S, will provide laboratory operations support including instrument repair and maintenance (b)(4) months)

(b)(6), (b)(3) 7 U.S.C. § 8401 will be responsible for growing and maintaining cell lines for supplying the biological assays. (b)(4) months)

Other Direct Costs: $200418 in YR3 has been budgeted for the purchase of biochemical supplies and reagents such as tips, microtiter plates, buffers, media, cells and detection reagents (i.e. Cell Titer Glo). Also, $99,000 in YR3 has been requested for the HTS service center charge at a rate of $300/hour for robot usage to prepare compound and assay plates. This charge includes service contracts, depreciation for the automation equipment, and regular QCing of the equipment. A BSL3 facility charge in the amount of $53,280 is also budgeted in YR3. There is a travel request of $2500 for Dr (b)(6), (b)(3) 7 U.S.C. § 8401 and (b)(6), (b)(3) 7 (b)(6), (b)(3) 7 to attend the annual CETR meeting.

A. COMPONENT COVER PAGE

Project Title: Medicinal Chemistry and Lead Development Core - Core C

Component Project Lead Information:

Pathak, Asish

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The primary goal of the MCLDC is to provide hit-to-lead analysis, synthetic chemistry, structure-activity relationship (SAR) data and analysis, computational support, and lead optimization chemistry to further the AD3C's mission of developing new broad-based therapeutics for the treatment of infections caused by emerging pathogens. In this role, the MCLDC, in conjunction with the Screening Core (SC; Core B), will be the central focus of the translational research component of the program. As the SC optimizes the novel assays developed by various Research Projects, and subsequently prosecutes the screening campaign, it will be the function of the MCLDC to assess the quality of the hit compounds that emerge, and ultimately to convert novel, tractable hits into potential clinically useful drugs with optimized biological and biophysical properties.

The Specific Aims of Core C, which remain unchanged, are:

Aim 1: Optimize screening hits identified through the primary HTS, dose-response, secondary assays, and counterscreens to identify compounds with the activity, selectivity, and pharmacokinetic properties to warrant animal testing For example, a typical compound that meets these criteria would have free plasma concentrations in the mouse (or rat, when administered IP, SC or PO) that exceeds the EC50 of the compound's primary activity by 2-5 fold for a period of time to be determined by the in vivo model used and associated in vitro data. The MCLDC will be responsible for all phases of optimization, scale-up, and submission to the SC for testing in the primary SAR screen as well as providing samples to the Center's participants All newly synthesized compounds will be fully characterized using standard spectroscopic and chromatographic tools (HPLC, LC/MS, NMR, MS, and elemental analysis as appropriate) In addition, the MCLDC will be responsible for performing a freedom to operate analysis as well as coordinating the filing of patent applications relating to new compounds.

Aim 2: Provide integrated informatics support including compound tracking, data capture, data analysis, and data storage, backup, and retrieval For each assay an appropriate Protocol ID will be assigned to track data relating to the informatics operations. For each compound synthesized we will import structures and assign a unique identifier (Dotmatics registration database). This number will be used throughout the Center to track compounds and the associated data.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Core C B.2.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

B.6.What do you plan to do for the next reporting period to accomplish the goals?
During Grant Yr3, we will continue the hit-to-lead chemistry approaches on one chemical series in each of the CHIKV, VEEV and influenza projects and on two chemical series for the SARS project We will also initiate any follow-up activities on any new chemical series that may be identified via the HTS screening data for DENV, CHIKV, VEEV and WNV to be completed by early Yr3. These hits will be prioritized for follow-up chemistry after reconfirmation in the SAR and viral load reduction assays
In Yr3, new screening results will be available from the antiviral assays currently under development within the four Research Projects, or already ongoing within Core B. Compounds identified as hits will undergo the usual triage before any re-synthesis or reconfirmation activities are initiated. The exact order and timing of follow-up is dependent on the HTS schedule of Core B; although at this time, it appears that the CHIKV and VEEV viruses will be the first assays to be completed In Yr3, we anticipate initiating chemistry on at least one new series from the CHIKV and VEEV screen and at least one additional series from DENV screen.

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

As described in our original grant application, while awaiting screening results from the assays being developed by the four AD3C Research Projects, Core C began hit follow-up using data from previously run screens targeting these same viruses. These previous screens were run for other programs that often did not include significant resources for hit follow-up. Activities included synthetic medicinal chemistry, computational chemistry, and purchase of commercial analogs of selected hit compounds. Hit compounds were triaged using the Pan Assay Interference Compounds (PAINS) filter and interesting compounds that remained were acquired either commercially or by re-synthesis. These compounds were provided as fresh new samples to the four Research Project labs for confirmation of their antiviral activity by testing in other virus strains, for cytotoxicity, and in some cases, in preliminary mechanism of action studies.

Specifically, In Yr 1, Core C analyzed data from five previous screening assay campaigns: a 347,000-compound library for the Dengue virus (DENV); a 288,000-compound library for the West Nile virus (WNV); a 384,000-compound library for the Venezuelan equine encephalitis virus (VEEV); a 102,000-compound library for the SARS coronavirus; and a 25,000-compound library for the influenza A virus. The acquisition of fresh samples and re-synthesis of non-available compounds was completed in the first quarter of Yr 2. These compounds were also sent for re-confirmation in Research Project Labs and were added to the 300K+ AD3C library set for future high-throughput screening (HTS). A total of 173 prior screening hits were selected for potential follow-up, of which 116 were readily available commercial samples that were distributed to the Research Projects for testing in antiviral activity and cytotoxicity assays. An additional 23 screening hits that were not commercially available were re-synthesized for testing. Results of these studies are provided in Section G (Core Specific Information).

For the VEEV and CHIKV projects, we initiated the synthesis of selected hits to evaluate the potential and quality of each hit as a potential lead chemical series for further development. At least 150 new and rationally designed analogs of one lead series for CHIKV were synthesized and screened for antiviral activity and cytotoxicity at Research Project 2 lab. In VEEV project, 70 new analogs of a lead series (that was previously identified in Yr 1.) were synthesized or commercially acquired and screened in antiviral and cytotoxicity assays at Research Project 2 lab. The pharmacokinetic properties of selected compounds within each series were also determined. Results of these studies are provided in Section G (Core Specific Information).

For the Influenza project (Research Project 4), one lead series was tested and showed modest antiviral activity against H1N1, H5N1, and H3N2 serovars. On the basis of these results, hit follow-up synthesis on this series was initiated to evaluate the potential and quality of this chemical series. Thus far, approximately 32 analogs within this chemical series have been synthesized or commercially acquired. Details of antiviral activity, cytotoxicity and *in vitro* ADME profiles on the series are provided in Section G (Core Specific Information).

During the 1st quarter of Yr 2, new high-throughput screening SARS hits that were identified from a 300K+ AD3C library set were triaged after PAIN filtration and prioritized based on their antiviral activity and cytotoxicity. This resulted in 4 potential lead series. Compounds active (via antiviral dose response) were then tested in new NanoLuc (NL) assay, the structure activity relationship (SAR) assay as well as in the cytotoxicity assay, both using VeroE6 cells. Approximately 230 compounds were synthesized across 4 different lead series and tested in NL SAR assay. Results of these studies are provided in Section G (Core Specific Information).

The results from the high-throughput screen of a 300K+ AD3C library set against the dengue virus yielded 11 active compounds after filtering through PAIN's filter. New samples of these hits were acquired commercially or re-synthesized and are being supplied to the Research Project lab for re-confirmation of antiviral and cytotoxicity activity. Simultaneously, these compounds are also being evaluated for their antiviral activity in a mirror ball (MB) SAR assay. Confirmed hits will be further evaluated as potential chemical leads for this program.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

File uploaded: Core C C.5.a.pdf

C.5.b Resource sharing

File uploaded: Core C C.5.b.pdf

C.5.a. *Other products: e.g. audio or video, research material such as cell lines, animal models, etc.).*

Limited quantities of most synthetic compounds will be made available to qualified individuals for research purposes once the pertinent data has been published.

C.5.b. *Resource sharing.*

As stated in the original Resource Sharing Plan for Core C (p. 334 of application), and as noted above (C.5.a), once published, and while compound supplies last, we will make research samples of synthetic compounds available to the scientific community for use as chemical probes and for other biological studies. Moreover, we fully expect all biological and chemical data to be published in scientific manuscripts after appropriate patent protection is in place. At this early stage of chemistry, there is no specific resource sharing to report.

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

F 2. Actual or anticipated challenges or delays and actions or plans to resolve them.

Core C was designed to escalate activities in Yr 3 in order to accommodate hit-to-lead phases in all four projects. Core B begin SAR assay implementation and screening of rationally designed small sets of analogs of confirmed hits. HTS screens on 300K+ AD3C screening library for SARS and DENV viruses are complete and will be completed for the CHIKV and VEEV viruses by the end of Yr 2. The HTS screens on WNV and Influenza will follow soon after and be completed by first quarter of Yr 3 The activities of Core C are dependent upon the completion of the high throughput screens for these viruses. If there is a lag period for testing compounds in the SAR assays (Core B) for any of these viruses, compounds can also be evaluated in the various Research Project 1–4 labs Core B has already established and set-up SAR assays for testing compounds against the SARS and dengue viruses Moving forward, we will continue to prioritize chemical series for each of the target viruses. To date, we have identified 6 chemical series for lead optimization In addition, it is anticipated that additional chemical series will be identified from data generated by the various Research Projects and from the new high throughput data from the ongoing assay screens which will support our completion of the Research Plan during the next reporting period.

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RPPR - Core-5324

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Asish		Pathak	PhD	Project Lead	(b)(4) (b)(6)				51,032.00	22,862.00	73,894.00
2.		Corinne		Augelli-Szafran	PhD	Co-Project leader					32,608.00	14,608.00	47,216.00
3.		Mark		Suto	PhD	Co-Project leader					16,304.00	7,304.00	23,608.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name·

Total Senior/Key Person **144,718.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
10	6 Chemist, 2 Scientist, 1 PK Technician, 1 Project Management	(b)(4)			397,566 00	178,110 00	575,676 00
10	**Total Number Other Personnel**					**Total Other Personnel**	**575,676.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**720,394.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0069005260000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	5,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**5,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0 00
2. Stipends		0.00
3. Travel		0 00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Southern Research Institute

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

F. Other Direct Costs	Funds Requested ($)*
1 Materials and Supplies	318,021.00
2 Publication Costs	0 00
3 Consultant Services	0.00
4. ADP/Computer Services	0.00
5 Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Hazardous waste	3,250.00
Total Other Direct Costs	**321,271.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**1,046,665.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. OH - Salaries + Benefits	120.0	720,394.00	864,473.00
2. G&A - Total Direct Cost + OH	20.0	1,911,138.00	382,228.00
3. CFC - Total Direct Cost + OH	1.0	1,911,138.00	1,911.00
4. CFC - Salaries + Benefits	4.6	720,394.00	33,138.00
	Total Indirect Costs		**1,281,750.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**2,328,415.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Budget Justification Yr 3 - Chemistry.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Program Director/Principal Investigator (Last, First, Middle):

Budget Justification — Medicinal Chemistry and Lead Development Core

Ashish K. Pathak, Ph.D. will serve as Core Leader of the Medicinal Chemistry and Lead Development Core. He has over 26 years of experience in medicinal and synthetic organic chemistry, including significant experience in anti-infective and vaccine adjuvant design. He has established himself as an independent researcher here at SR and also led research group as an Assistant Professor in the Department of Chemistry, Western Illinois University before joining SR. Currently, he also manages the high-throughput parallel synthesis group and is involved in several internal drug discovery projects as a supervisor in medicinal chemistry. He has extensive experience in early lead discovery to lead optimization and in all aspect of a medicinal chemistry program. He has been PI of two R21 NIH funded programs in the area of viral vaccine adjuvant discovery. In collaboration with the PIs and other Co-Investigators in the center, he will oversee all aspects of the medicinal chemistry effort, including hit triage and synthetic target selection; target design, synthesis, and the planning of synthetic routes; compound characterization; structure-activity analysis and interpretation of biological results; cheminformatics and molecular modeling; report, manuscript, and patent preparation; and overall project management of lead optimization chemistry. Dr. Pathak will devote (b)(4) months to the Core during Year 3.

Mark J. Suto, Ph.D. (Vice President, Drug Discovery Division) and Corinne E. Augelli-Szafran, Ph.D. (Director, Chemistry Department) will serve as Co-Core Leaders of the medicinal chemistry core for the proposed project. Each has approximately 30 years of drug discovery experience across multiple therapeutic areas, including all steps in discovery and development, with a focus on discovering, developing, and advancing new compounds to the clinic in an effective and efficient manner, ensuring that all of the needed data for regulatory filings are properly gathered. In addition to experience in early lead identification and discovery, target validation, and lead optimization, Dr. Suto has served on clinical development teams and managed the preparation of drug product for clinical trials. For the current project, Drs. Pathak, Suto, and Augelli-Szafran will work as a team in the selection of hit compounds to move forward, and in the subsequent selection of lead candidates. They will be involved in all aspects of the medicinal chemistry program, including the design of optimal development strategies for individual lead candidates. Dr. Suto will devote (b)(4) month and Dr. Augelli-Szafran will devot (b)(4) months to the Medicinal Chemistry and Lead Development Core in Year 3.

Wei Zhang, Ph.D. (Research Scientist, Chemistry Department) has a broad background in development and application of computational methods for the modeling and understanding of biological systems. He holds a Ph.D. in computational chemistry and had postdoctoral training in structural biology and computer-aided drug design. For this project, Dr. Zhang will provide computational chemistry support for each hit follow-up and lead optimization project, including cheminformatics, clustering analyses, and hit triage filtering; calculation of molecular properties; SAR analysis and model building (pharmacophore, homology, etc., as appropriate to the given project); searches for commercial analogs of hit compounds; and virtual screening against selected viral target proteins. He will also assist in the preparation of appropriate reports, manuscripts, and patents. Dr. Zhang will devote variable effort as needed, depending upon the number of active projects, beginning with (b)(4) (b)(4) months in Year 3.

Kaleem Ahmed, Ph.D., Nikhil Madadi, Ph.D., Saibal Chakraborty, Ph.D., and two to be hired (TBH) chemists, will be directly responsible for day-to-day synthetic activities in the laboratory. They will perform compound syntheses, compound scale-up, library research, and assist in target selection and the design of analogs, the planning of synthetic methodology, compound characterization, data analyses, cheminformatics and molecular modeling, and report and manuscript preparation. Each of these chemists has several years of post-doctoral research experience in the design and preparation of compounds of many types, and in the analysis of structure-activity relationship data. Each will devote (b)(4) to this project over the duration of the grant award, performing hit to lead chemistry on active series against different viruses for Projects 1-4 and gram scale synthesis on lead molecules for animal studies. In Grant Year 3, Drs. Ahmed, Madadi and Chakraborty, and two to be hired new Ph.D. chemists will devote (b)(4) months. The chemistry resources allocated originally for Tranzyme (Cyclenium) to develop unique macrocycles, which is no longer available for collaboration to this center, will be utilized in hit to lead chemistry efforts in house at SR to develop additional

lead compounds resulting from highthroughput screens.

David Poon, Chemist, Supervisor of Compound Management in Chemistry Department at SR. He has extensive experience in managing in-house synthesized compounds and commercial libraries. He will provide integrated informatics support including compound tracking, data capture, and data storage, backup, and retrieval. He is incharge of maintaining our in-house Dotmatics registration database which is used extensively in this program to assign a unique identifier to each compound synthesized or acquired commercially. This identifier number is used throughout the Center to track compounds and any associated data. He is also responsible for sample preparation and distribution to different project teams and to Core B. He will devote (b)(4) (b)(4) months to this program in Year 3.

(b)(6) (b)(3) 7 U S C § 8401 MS, PMP has five years of experience in coordinating and managing research projects in Drug Discovery Division at SR. She will work with Dr. Suto and the other project leaders to ensure a timely and efficient delivery of Core services to the overall program and will devote (b)(4) months in Year 3 to the program.

In Grant Year 3 (and variable in subsequent years), we have allocated $60,000 for the purchase of commercial analogs of hit compounds in order to generate preliminary structure activity relationship (SAR) data. This estimate is based on at least three-four highthroughput assays being completed by the end of Year 2, each generating four scaffolds for follow-up analoging, and 50 commercial analogs being purchased (10-20 mg quantities) for each scaffold at approximately $100/compound.

Each of the laboratory synthetic chemists has been allocated a reagent budget of ~$40,000 per year per FTE, based on our previous experience in these types of lead optimization chemistry programs. This budget covers starting materials, specialized reagents, solvents, chromatography supplies, resins and solid-phase synthesis supports, glassware, plasticware, and other disposables, as well as spectroscopy and compound characterization expenses. The supply costs for synthetic FTEs are proposed to $200,000 for Grant Year 3.

Approximately $3,250 is allocated for hazardous waste disposal for Grant Year 3.

Donghui Bao, Ph.D., is a Research Scientist and supervisor of the bioanalytical laboratory in the Chemistry Department at SR. He has extensive experience in developing and validating efficient bioanalytical methods for quantitative analysis of novel pharmaceuticals, metabolites, and endogenous compounds for use in clinical and non-clinical research, including working knowledge of GLP regulations. He also has expertise in quantitative bioanalytical validation including SPE, HPLC, and MS/MS development and optimization; operation, maintenance, and calibration of LC-MS/MS instrumentation and Rapid Trace SPE instrumentation; research involving animal models including oral, IV, and IM dosing; aseptic cell culture techniques including the use of cultured and freshly isolated hepatocytes; and the handling and analysis of radioisotopes by Liquid Scintillation and Gamma Counting (3H, 14C, 51Cr, 125I). Dr. Bao will be responsible for overseeing PK/ADME studies in all lead optimization projects. He will devote (b)(4) months to this project.

One technician for animal work will devote 40 hrs/PK study/compound for pharmacokinetic and toxicological profiling of potential drug candidates emerging from the lead optimization program under the direction of Dr. Bao. $58,021 annually has been allocated to covers PK/ADME supplies and animal costs for these studies.

$5,000 annually is allocated for the Core Leader and Co-Core Leaders to attend the required NIAID CETR Program Meeting.

Note: ICD rate for CFC Total Direct Cost + OH is 0.10%; online form does not allow less than 1%

A. COMPONENT COVER PAGE

Project Title: Project 1.2 Identification and Development of Anti-Flavivirus Lead Drug Candidates

Component Project Lead Information:

Diamond, Michael S

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Rationale 2'-O-MTase as a target for small molecule screens. Type I interferon (IFN) cell-intrinsic antiviral defenses protect against many virus infections by signaling host blockade of viral translation, transcription, and replication, thus limiting spread and pathogenesis. Cellular mRNA of higher eukaryotes and many viral RNA are methylated at the N-7 and 2'-O positions of the 5' guanosine cap by specific nuclear and cytoplasmic MTases, respectively Whereas N-7 methylation is essential for RNA translation and stability the function of 2'-O methylation and its role in virus infection remained uncertain since its discovery 35 years ago until recently Studies by members of our group have shown that 2'-O MTase activity of flaviviruses, coronaviruses, and poxviruses promotes viral evasion of Ifit family of genes, a group of IFN-stimulated innate immune effector proteins. Viruses lacking 2'-O MTase activity were attenuated in wild type primary cells and immunocompetent animals but were rescued in cells and mice lacking Ifit1 gene expression. This data is consistent with a model in which 2'-O methylation of the 5' cap of viral RNA subverts innate host antiviral responses through escape of IFIT-mediated suppression, and suggest an evolutionary explanation for 2'-O methylation of cellular mRNA: to distinguish self from non-self RNA The fact that cytoplasmic viruses cannot use nuclear host 2'O MTases and therefore encode their own viral 2'-O MTases attests to their evolutionary success against their hosts. Nonetheless, given that host 2'-O methylation of cellular mRNA largely occurs in the nucleus, pharmacological strategies that specifically disrupt cytoplasmic viral 2'-O MTase activity could represent a novel class of broad-spectrum antiviral therapy against a number of globally relevant human pathogenic viruses that replicate exclusively in the cytoplasm, including flaviviruses
Goal Identification of compounds that inhibit viral 2'-O MTase activity and sensitize flaviviruses to the antiviral effects of Ifit1 Compounds that inhibit WNV infection in Ifit1-expressing cells will be tested across a full dose-range for their activity in T-antigen transformed MEFs that ectopically express Ifit1. Small molecules that specifically block 2'-O MTase activity should have little or no inhibitory effect in Ifit1-/- cells but should function specifically in isogenic cells expressing Ifit1. Compounds that show this dependence on Ifit gene expression for inhibition of viral replication will be further analyzed as potential inhibitors of viral 2'-O MTase activity Again, these 'hits' should have no effect on WNV-NS5-E218A, which already lacks 2'-O MTase activity. As final proof of their mechanism of action, lead compounds that sensitize flaviviruses to the effects of Ifit1 in cell culture will be tested in vivo for their ability to differentially inhibit flaviviruses in Ifit1+/+ and Ifit1-/- mice.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

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B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

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B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

B.6. Plans for next year.
With our new doxycycline inducible 293T cell line, SRI will perform a HTS to identify small molecule lead candidate hits that specifically target 2 -O methyltransferase activity. The Diamond laboratory will validate such hits by performing full multi-step growth curve analysis in parallel with cytotoxicity studies This will allow a selected subset of compunds to be tested against other cell types and flaviviruses using WT viruses in cells lacking or expressing IFIT1.

In addition to this, we have recently identified a new host target gene, SPCS1, as a candidate required gene for infection by all flaviviruses This gene represents a non-canonical signal peptide-processing pathway that flaviviruses but not other viruses use This year, we will design a subviral particle transfection assay for high-throughput screening of small molecule inhibitors of this pathway.

A. Rationale. 2'-O-MTase as a target for small molecule screens. Type I interferon (IFN) cell-intrinsic antiviral defenses protect against many virus infections by signaling host blockade of viral translation, transcription, and replication, thus limiting spread and pathogenesis. Cellular mRNA of higher eukaryotes and many viral RNA are methylated at the N-7 and 2'-O positions of the 5' guanosine cap by specific nuclear and cytoplasmic MTases, respectively. Whereas N-7 methylation is essential for RNA translation and stability the function of 2'-O methylation and its role in virus infection remained uncertain since its discovery 35 years ago until recently. Studies by members of our group have shown that 2'-O MTase activity of flaviviruses, coronaviruses, and poxviruses promotes viral evasion of Ifit family of genes, a group of IFN-stimulated innate immune effector proteins. Viruses lacking 2'-*O* MTase activity were attenuated in wild type primary cells and immunocompetent animals but were rescued in cells and mice lacking *Ifit1* gene expression. This data is consistent with a model in which 2'-O methylation of the 5' cap of viral RNA subverts innate host antiviral responses through escape of IFIT-mediated suppression, and suggest an evolutionary explanation for 2'-O methylation of cellular mRNA: to distinguish self from non-self RNA. The fact that cytoplasmic viruses cannot use nuclear host 2'O MTases and therefore encode their own viral 2'-O MTases attests to their evolutionary success against their hosts. Nonetheless, given that host 2'-O methylation of cellular mRNA largely occurs in the nucleus, pharmacological strategies that specifically disrupt cytoplasmic viral 2'-O MTase activity could represent a novel class of broad-spectrum antiviral therapy against a number of globally relevant human pathogenic viruses that replicate exclusively in the cytoplasm, including flaviviruses.

B. Goal. Identification of compounds that inhibit viral 2'-O MTase activity and sensitize flaviviruses to the antiviral effects of Ifit1. Compounds that inhibit WNV infection in Ifit1-expressing cells will be tested across a full dose-range for their activity in T-antigen transformed MEFs that ectopically express Ifit1. Small molecules that specifically block 2'-O MTase activity should have little or no inhibitory effect in *Ifit1*$^{-/-}$ cells but should function specifically in isogenic cells expressing Ifit1. Compounds that show this dependence on Ifit gene expression for inhibition of viral replication will be further analyzed as potential inhibitors of viral 2'-O MTase activity. Again, these 'hits' should have no effect on WNV-NS5-E218A, which already lacks 2'-O MTase activity. As final proof of their mechanism of action, lead compounds that sensitize flaviviruses to the effects of Ifit1 in cell culture will be tested *in vivo* for their ability to differentially inhibit flaviviruses in *Ifit1*$^{+/+}$ and *Ifit1*$^{-/-}$ mice.

C. Progress. Generation of inducible Ifit1-expressing MEFs.

1. Derivation of SV2-transformed MEFs. Murine embryonic fibroblasts (MEFs) were derived from 15 day-old *Ifit1*$^{-/-}$ C57BL/6 embryos and cultured *in vitro* for three passages. T-150 flasks of confluent MEFs were transfected with the pSV2 plasmid encoding the SV40 polyoma virus large T antigen. Transfected cultures were incubated at 37°C and monitored for cell death with media being replaced ever three days to remove cellular debris. When colonies of transformed cells became visible (roughly 2 weeks), the cells were replated and then passaged ten additional times before being frozen in liquid nitrogen.

2. Production of FKBP-mIFIT1 inducible MEF line. SV2-immortalized Ifit1$^{-/-}$ MEFs (p20) were transduced with the lentiviral vector pMD144-FKBP-mIFIT1 (kind gift from H. Malik) in 6-well plates. The FKBP de-stabilization domain (DD) is appended to the N-terminus of IFIT1 and results in rapid degradation when expressed in mammalian cells; this allows transfection of the IFIT1 construct with low basal levels of expression in Ifit1$^{-/-}$ MEFs. However, The FKBP-derived destabilizing domains are blocked by the addition of small molecules (e.g. Shield1); inclusion of Shield1 ligand in the medium stabilizes a DD-tagged protein of interest in a predictable and dose-dependent manner.

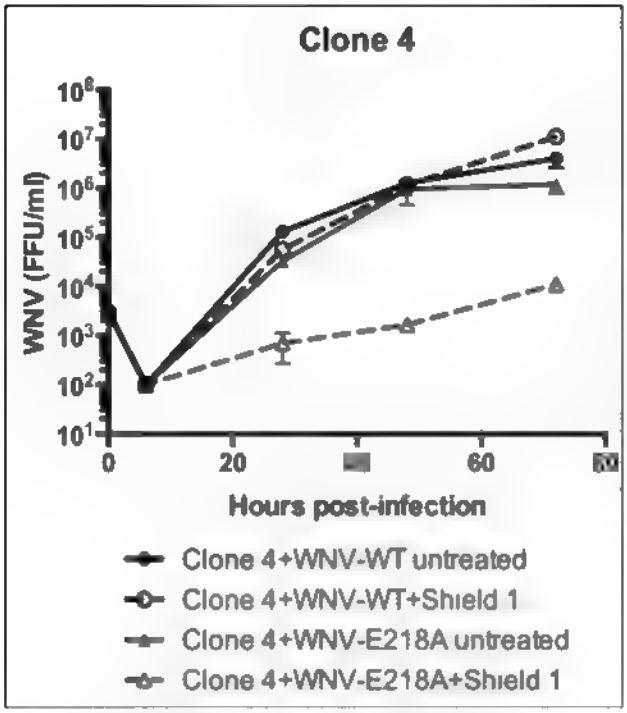

Figure 1. Induction of IFIT1 in Ifit1$^{-/-}$ MEFs using Shield1 ligand results in selective inhibition of WNV strains lacking 2'-O methylation (WNV-NS5-E218A). At baseline, ectopically expressed IFIT1-DD is rapidly degraded. Addition of Shield1 stabilizes expression of IFIT1 and confers an antiviral effect against WNV-NS5-E218A

To generate this inducibe cell line, cells were transduced with the lentivirus and cells were selected with puromycin. After three days of drug selection, the culture media was replaced and the concentration of puromycin was reduced to maintain FKBP-mIFIT1 gene expression. Individual colonies were then picked and transferred to 6-well plates for expansion. Individual clones were then assessed for FKBP-mIFIT1 expression by flow cytometry (using an anti-IFIT1 monoclonal antibody) and mIFIT1 function by assessing replication of WNV-WT and WNV-NS5-E218A in the presence and absence of Shield-1 treatment.

In the absence of Shield1, Ifit1 expression was low and no difference in viral infection was observed in viruses having (WNV-WT) or lacking (WNV-NS5-E218A) 2'-O methylation (**Fig 1**). In the presence of Shield1, Ifit1 expression was rapidly increased, and only viruses lacking 2'-O methylation became sensitized to the antiviral effects of Ifit1. Thus, an inducible cell line was generated and sent to SRI for subsequent HTS.

3. Production of doxycycline-inducible 293T cell line expressing mouse or human IFIT1. As an independent cell for high-throughput screening and validation, we have generated a 293T cells that expresses IFIT1 genes under a doxycycline inducible promoter. At baseline, IFIT1 is not expressed but with the addition of 1 μg/ml of doxycycline, IFIT1 is expressed as judged by flow cytometry (**Fig 2, left**). This level of IFIT1 expression is sufficient to inhibit infection of WNV strains lacking 2'-O methylation (WNV-NS5-E218A) but does not inhibit wild-type parent viruses (**Fig 2, right**). These cells have been shipped to Southern Research Institute for further high throughput screening.

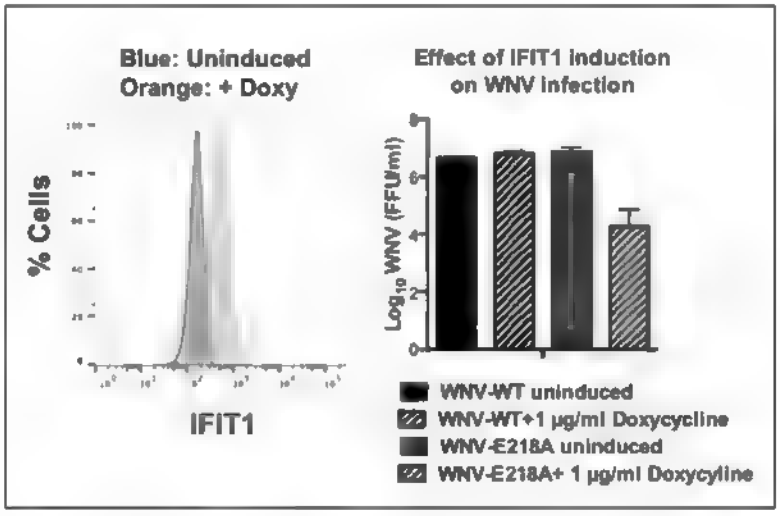

Figure 2. Induction of IFIT1 in 293T cells with doxycycline. Left. Flow cytometry histograms. Right. Viral yield assay at 48 h

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

The Office of Postdoctoral Affairs (OPA) encourages all postdocs to complete an individual development plan, and recommends using the tool myIDP hosted by Science Careers. New postdocs are introduced to IDPs and the myIDP tool during orientation and workshops are offered throughout the year. OPA recommends that faculty review Individual Development Plans with postdocs at their annual review. IDPs should be reviewed and updated at least annually.

We recognize that postdocs need both information and opportunities to explore the variety of career outcomes pursued by our alumni. OPA has an Education Coordinator and the University employs a full-time career strategist to provide career and professional development training along with Career Talks for postdocs.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

File uploaded: Project 1 Diamond C.5.a.pdf

C.5.b Resource sharing

NOTHING TO REPORT

C.5a. Other products.

We have generated the doxycycline inducible 293T cell that expresses IFIT1. Upon publication, we will deposit this cell line at BEI Resources (ATCC) for use by the greater scientific community.

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0685522070000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Washington University

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Michael		Diamond	MD	Project Lead	(b)(4) (b)(6)				9,165.00	1,915.00	11,080.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **11,080.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
2	Post Doctoral Associates	(b)(4)			48,510.00	14,101.00	62,611.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Sr. Research Technician				5,153 00	1,780 00	6,933 00
3	**Total Number Other Personnel**					**Total Other Personnel**	**69,544.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**80,624.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0685522070000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: Washington University

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0 00
2. Stipends		0.00
3. Travel		0 00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0685522070000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: Washington University

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

F. Other Direct Costs	Funds Requested ($)*
1 Materials and Supplies	79,208 00
2 Publication Costs	2,000 00
3 Consultant Services	0.00
4. ADP/Computer Services	0.00
5 Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Equipment service contracts	2,500.00
9. Machine Shop/Computer Maintenance	2,000.00
10. Glassware/BSL3 Waste Disposal	3,000.00
Total Other Direct Costs	**88,708.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**172,332.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC On Campus	52.5	172,332.00	90,474.00
		Total Indirect Costs	**90,474.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**262,806.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Budget Justification Whitley Diamond-Year 2.pdf (Only attach one file.)

RESEARCH & RELATED Budget (F-K) (Funds Requested)

BUDGET JUSTIFICATION

PERSONNEL:

Michael S. Diamond, M.D., Ph.D., Co-Investigator. Professor of Medicine, Molecular Microbiology Pathology and Immunology at Washington University School of Medicine. Dr. Diamond will devote (b)(4) months to this project and receive (b)(4) months of salary support. He will be responsible for the design and oversight of all investigations occurring in his laboratory. He will actively participate in the planning and execution of experiments, as well as in the generation of progress reports and manuscripts.

Kyle Austin, Ph.D. Post-doctoral Research Associate. Dr. Austin is a post-doctoral fellow that has been in the Diamond lab for more than six months. He has significant experience in flavivirus biology and will perform studies in cell culture to test the effects of lead hits on WNV and other viruses. He will devote ~(b)(4) months of time to this project and derive (b)(4) months salary from it. He will be responsible for interpreting data and troubleshooting technical problems in consultation with the principal investigator.

Jennifer Govero, PhD. Sr. Research Associate. Dr. Govero has significant experience (~7 years post-Ph.D.) in virology, immunology, and animal models of disease. She will devote (b)(4) months of her time to this project and derive (b)(4) months of her salary accordingly, beginning in FY2. One of her primary roles will be participating in the testing of small molecules in mice. She also will work with Dr. Austin on some of the virological studies in characterizing the lead molecules in vitro and in vivo.

Michelle Noll, Animal Technician. This project requires a significant amount of animal work associated with breeding of Ifit1 KO mice, genotyping, and conducting animal experiments. Ms. Noll, our experienced animal technician, will be responsible for animal husbandry under the oversight of Drs. Govero and Diamond. She will devote (b)(4) months to the project and receive (b)(4) months of salary support in FY 3-5.

EQUIPMENT: No new equipment is needed.

SUPPLIES:

Tissue culture ($10,000). With this project, there will be a considerable amount of tissue culture associated with cell-based validation of small molecule hits and target gene identification. The funds requested will be used for media preparation, growth additives, primary cell culture, serum, antibiotics, plasticware (disposable pipets, pipetman tips, flasks, tubes, cryogenic vials, filtration flasks, sterile bottles).

Molecular Biology Reagents ($10,000). This amount has been budgeted for reagents for molecular cloning (restriction enzymes), proteases, transfection (liposomes, electroporation cuvettes), plasmid DNA and RNA purification kits, electroporation, vectors, bacterial culture supplies, and DNA/RNA/protein electrophoresis. .

Chemicals ($4,112). This amount has been budgeted for general chemical supplies including buffers, salts, organic solvents, acids, bases, and detergents.

Immunochemical and Reagents ($5,000). This has been budgeted for the direct labeling of antibodies with different fluorophores, for secondary reagents for ELISA, and for intracellular immunofluorescence studies. Some validation screening in different cell types will be performed using immunofluorescence as the readout. This budget also includes time on a shared confocal microscope (~$100/hour).

Real-time RT-PCR ($5,000). We have developed a sensitive and reproducible quantitative real-time RT-PCR for assessing flavivirus replication using an ABI 7000 Sequence Detection instrument. This assay has become our standard for RNA quantitation and will be used to measure viral RNA levels in serum and in tissue and cells. RT-PCR reagents, primers, Taq-Man probes cost approximately $1 per well. In addition, for most samples, a ribosomal or actin RNA control is run for normalization purposes. Also, some of the in vivo studies will use RNA-based methods for quantitation.

Flow Cytometry Reagents and equipment time ($2,500). This amount is for reagents associated with running of the flow cytometer including calibration, buffers, controls, and software licenses. The flow cytometry will be used for screening and validation small molecules that restruct WNV infection.

DNA Oligonucleotides ($2,000). We will use the supplier of the core facility at Washington University which charges approximately $0.20 per base. These oligonucleotides will be used for cloning and sequencing.

Liquid N2/CO2 ($1,000). This amount is budgeted for liquid N2/CO2 bottled gas and related tank rental fees.

Animal pharmacy/sentinal testing/dissection ($2,000 – *FY3-5*). This reflects the costs of anaesthetics and surgical tools for necropsy, and the charges for sentinel testing.

Animal Puchases, Breeding, and Housing ($37,596 – FY3-5). Beginning in FY3, the costs reflect the purchase and breeding for all prophylaxis and therapeutic experiments with small molecules. Also built into these costs are the purchase of Alzet osmotic pumps for drug delivery.

OTHER EXPENSES:

Equipment Service Contracts ($2,500). This money is budgeted for service contracts on all essential equipment to be used with this grant ELISPOT reader (for viral focus forming assays), our 96-well plate flow cytometer, and ABI 7500 TaqMan machine.

Other ($5,000). This money is budgeted for additional costs associated with operation (disposable gowns, gloves, boots, barrier tips) and usage of the BSL3 facilities along with waste disposal ($3,000). Funds are also budgeted for machine shop and computer maintenance ($2,000).

Publications ($2,000) we request support on publishing at least one manuscript per year ($2,000).

Travel ($3,000) Travel has been budgeted for one reverse site visit with NIH, one meeting with the Whitley/Nelson laboratories, and a scientific meeting to present data.

A. COMPONENT COVER PAGE

Project Title: Project 2.2 Inhibitors of Coronavirus Fidelity and Cap Methylation as Broadly Applicable Therapeutics

Component Project Lead Information:

Baric, Ralph S

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The overall goal of Project 2 is to identify inhibitors of two highly conserved CoV processes, replication fidelity and RNA capping, that are essential for SARS-CoV virulence and survival in vivo Multiple viral proteins and enzymatic activities are critical for these processes, including CoV 3'-to-5' exoribonuclease (fidelity, nsp14-ExoN) and 2'-O-methyltransferase (capping; nsp16-OMTase) activities. Consistent with the importance of these processes, we have shown that decreased replication fidelity and ablation of RNA capping through genetic inactivation of either ExoN or OMTase, respectively, results in replication competent viruses that are profoundly attenuated in vivo

Aim 1. To identify and develop inhibitors of CoV high-fidelity replication We will test the hypothesis that inhibitors of CoV high-fidelity replication will decrease viral fitness alone and in combination with RNA mutagens, and represent potent pan-CoV therapeutics. In part 1, we will identify ribonucleoside analogs that inhibit CoV replication, and define their mechanism of action High-throughput screening in part 2 will identify small-molecule inhibitors of CoV fidelity. In part 3 we will identify the viral protein targets of lead compounds, and determine their mechanism of fidelity impairment. In part 4, will we test highly efficacious compounds identified in parts 1 and 2 across the CoV family and viral platforms within this program.

Aim 2. To identify and develop inhibitors of CoV RNA capping activity. We hypothesize that small molecule inhibitors of essential CoV RNA capping components will profoundly increase CoV sensitivity to the host innate immune response through interferon-stimulated effectors In part 1 we will use targeted mutagenesis of known CoV capping components to define distinct mechanisms to increase CoV sensitivity to the host ISGs In part 2 we will examine the combined efficacy of known O-MTase inhibitors and type I IFN treatment against SARS-CoV, and perform a high-throughput screen for inhibitors of CoV RNA capping. In part 3 we will identify the viral protein targets and mechanism of action of lead compounds In part 4, lead compounds will be tested across the CoV family and specific viral platforms within this program

Aim 3. To chemically optimize and test the in vivo efficacy of CoV fidelity and RNA capping inhibitors We will test the hypothesis that inhibitors of CoV fidelity or RNA capping are highly attenuating in vivo and represent broadly effective CoV therapeutics. Compounds identified in Aims 1 and 2 will be chemically optimized for in vitro efficacy, selectivity, solubility, microsomal stability, and bioavailability at SR. Using these optimized compounds, in part 1 we will confirm the biological target(s) of lead fidelity and RNA capping inhibitors in vivo. In part 2 we will test the efficacy of lead compounds against mouse-adapted SARS-CoV in progressively stringent mouse models of acute and persistent human disease. Efficacy will be determined by monitoring respiratory function, morbidity and mortality, histology, and viral replication. In part 3 we will test for the development of drug resistance in vivo, and will determine the efficacy of lead compounds against MERS-CoV and other CoV family members.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded Project 2 Baric B2 Done 12.9 SD.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded Project 2 Baric B4 Done 12.7.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

The plans for Year 3 Project 2 can be found under the information for Project 2.1.

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

The accomplishments for Project 2 can be found under the information for Project 2.1.

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

One Postdoctoral Fellow is active in the project. At UNC, Individual development plans (IDPs) are generated on an annual basis. They are used for defining key objectives and goals for progress and for review on at least an annual basis. For this project, the IDPs will review specific goals relevant to the project. For postdoctoral fellows, these assist in analysis of progress in projects; in addition they help in career development. For IDPs, both biosketches and CVs are appended, so that it is possible to use these as learning tools.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Ralph		Baric	PhD	Project Lead	(b)(4) (b)(6)				16,497.00	4,244.00	20,741.00
2.		(b)(6) (b)(3) 7 U S C § 8401			PhD	Co-investigator					24,251.00	6,883.00	31,134.00
3.					PhD	Co-investigator					20,600.00	6,052.00	26,652.00
4.					PhD	Co-investigator					20,600.00	6,052.00	26,652.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **105,179.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	Research Technician, Research Specialist	(b)(4)			21,850.00	7,157.00	29,007.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**29,007.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**134,186.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 6081952770000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	6,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**6,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0 00
2. Stipends		0.00
3. Travel		0 00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The University of North Carolina at Chapel Hill

Start Date*: 03-01-2016 **End Date*:** 02-28-2017

F. Other Direct Costs	Funds Requested ($)*
1 Materials and Supplies	125,764 00
2 Publication Costs	2,000 00
3 Consultant Services	0.00
4. ADP/Computer Services	0.00
5 Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Deep Sequencing: Maintenance Contracts	15,000.00
9. Histology, flow cytometry	13,000.00
10. Animal per diem; shipping	8,421.00
Total Other Direct Costs	**164,185.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**304,371.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	52.0	304,371.00	158,273.00
		Total Indirect Costs	**158,273.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**462,644.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Year_3_Budget_Justification_Whitley CETR Baric.pdf (Only attach one file.)

RESEARCH & RELATED Budget (F-K) (Funds Requested)

Budget Justification Baric Project Whitley CETR Proposal Year 3

Personnel:

Ralph Baric, Ph.D., Principal Investigator (b)(4) months). Dr. Baric will supervise the overall direction of the animal research agenda of this highly interactive proposal. He will interact closely with Drs. (b)(6) (b)(3) 7 U S C § 8401 and Sheahan, and (b)(6) (b)(3) 7 U S C § 8401 to ensure steady progress during the course of the proposal, evaluate results and propose alternative experiments. Dr. Baric will be also be responsible for interacting closely with all research staff, holding regular laboratory meetings, communicating research findings with the (b)(6) (b)(3)7 S C § 8401 laboratory, writing progress reports and managing fiscal matters associated with the proposal. Given the extensive interaction and collaboration with Dr. (b)(6) (b)(3)7 U S C § 8401 in the past, he will also lead efforts to coordinate and promote research efforts with the groups. Dr. Baric will communicate his findings with Dr. (b)(6) (b)(3)7 U S C § 8401 on a regular basis via both conference calls and meetings between the two laboratories.

(b)(6) (b)(3) 7 U S C § 8401 **Co-Investigator** (b)(4) months). (b)(6) (b)(3)7 U S C § 8401 will oversee all select agent research in the facility. (b)(6) (b)(3) 7 will test select SARS-CoV and MERS-CoV mutants in primary culture models to evaluate drug candidates in the more advanced human in vitro model. In addition, (b)(6) (b)(3) 7 will perform viral passage studies in the presence of drug candidates to identify escape mutations that may arise. (b)(6) (b)(3) 7 will report findings regularly to Drs. Baric and (b)(6) (b)(3)7 U S C § 8401 as well as interfacing with the drug candidate manufacturers.

Timothy Sheahan, Ph.D. Investigator (b)(4) months). Dr. Sheahan has extensive BSL3 experience and has recently rejoined the Baric laboratory as a Research Assistant Professor. He will lead the in vivo drug testing with wild type virus portion of the project in collaboration with Dr. (b)(6) (b)(3)7 U S C. § 8401 as well as performing many of the in vivo experiments. He will be assisted by Dr. (b)(6) (b)(3) 7 U S C § 8401

(b)(6), (b)(3) 7 U S C § 8401 **Ph.D. Investigator** (b)(4) months). (b)(6) (b)(3) 7 U S C § 8401 will lead the in vivo testing of compounds with SARS-ExoN I experiments and the in vivo virus evolution experiments proposed in the application. (b)(6) (b)(3) 7 in collaboration with Dr. Sheahan will lead our efforts in studying the pathogenesis of SARS-CoV ExoN mutants, derivative ExoN evolved viruses, and conducting in vivo persistent infections/evolution experiments in animals. (b)(6) (b)(3) 7 has extensive experience working with ExoN I in mice and is skilled at assembling recombinant SARS-CoV viruses using classic recombinant DNA approaches or synthetic genomic approaches. (b) 6) (b)(3) 7 will work closely with Drs. Baric, (b)(6) (b)(3) 7 U S C § 8401 for experimental design and analysis and will oversee (b)(6) (b)(3)7 U S C § 8401 in conducting in vivo pathogenesis experiments during the duration of the grant.

(b)(6) (b)(3) 7 U S C § 8401 (b)(4) months). (b)(6) (b)(3)7 U S C § 8401 has extensive BSL3 experience and will assist with viral titration assays and BSL3 animal husbandry. (b)(6) (b)(3) 7 will also support (b)(6) (b)(3) 7 U S C § 8401 and Sheahan's research efforts as needed.

(b)(6) (b)(3) 7 U S C § 8401 (b)(4) months). (b)(6) (b)(3)7 U S C. § 8401 has extensive BSL3 experience and will assist with viral titration assays, daily BSL3 laboratory maintenance and BSL2 animal husbandry. (b)(6) (b)(3)7 U S C. § 8401 will also be responsible for purchasing supplies, maintaining stocks in the BSL3, and supporting (b)(6) (b)(3) 7 U S C § 8401 and Sheahan's research efforts as needed.

Fringe Benefits: Faculty/Staff: 22.471% Social Security and Retirement; $5,471/FTE Health Insurance. Post-doctoral Research Associates: 8.99% Social Security and benefits; $4,373/FTE Health Insurance.

Travel

Travel: ($6,000): Domestic Travel: Funds are requested for the Project Leader and staff to attend 2 scientific conferences and the annual CETR U19 meeting in Bethesda each year. This allows program faculty and fellows to communicate results, develop collaborations and share research interests.

Supplies:

Molecular Biology Reagents ($10,000/year) Assembling recombinant SARS-CoV and MERS-CoV requires large amounts of highly expensive restriction enzymes (e.g., BsmB1, etc.) and large amounts of DNA ligase. In addition, funds are requested for DNA markers, high quality T7 RNA polymerase, and protein and nucleic acid markers. As sequence confirmation is critical prior to assembly of full length genomic cDNA, funds are also requested to sequence modified genomic fragments following introduction of ExoNI mediated mutations.

Synthetic DNA ($5,500/year) Funds are requested for the purchase of synthetic DNA fragments which are primarily purchased from small biotec companies like Blue Heron or Bio Basic Inc. at costs of about $0.35/base. Our budget allows for ~40,000-bp of synthetic gene synthesis/yr, sufficient for our needs over the course of this project and will allow for the rapid assembly of recombinant viruses bearing different ExoNI derived mutations.

BL3 Protective Gear ($13,000/year) Personnel wear powered air purifying HEPA filtered breathing apparatuses, wear tyvek suits, tyvek aprons, hoods, booties and are double gloved when entering the BSL3 facility. These materials are expensive as the HEPA, organic chemical filters and even batteries must be replaced every ~6 months, and the tyvek suits are disposable. Moreover, the PAPR (powered air breathing apparatus) are expensive and must be replaced every ~2 years from normal wear and tear, and daily contact with EPA disinfectants. Personnel use high quantities of disinfectants like ethanol, Clorox and other EPA approved disinfectants in maintaining a safe working environment in the BSL3. Personnel spray down tyvek suits, etc. with alcohol or related disinfectants in the process of deconing and leaving the BSL3 facility. All materials that leave the BSL3 must be disinfected, packaged in disinfected, sealed containers, which are disinfected prior to removal from the BSL3 facility. In addition, funds are requested to help defray costs associated with the decontamination and maintenance of the BSL3 laboratory each year.

Miscellaneous ($4,264/year) Monies are requested to purchase glassware, pipettes, etc. used in day to day virologic and cell culture procedures as well as in growing, titering and characterizing virus growth in vitro. Funds are also requested to purchase chemicals, reagents, paper products, gloves, micropipetors, autoclave supplies, plastic tips, water baths, and other small equipment items that typically have short half lives in laboratory settings.

Computer Supplies ($1,000/year) Funds are requested for project specific computer and software upgrades over the course of the proposal.

Tissue culture ($40,000/year) Funds are requested to purchase mature human airway epithelial cell (HAE) cultures for drug testing assays. Each culture is $100. We anticipate requiring 300 HAE cultures. In addition, we are requesting funds to purchase cell culture supplies and plasticware to perform virus plaque assays and general tissue culture work.

Immunology Reagents ($7,000/year) Funds to purchase supplies for flow cytometry and ELISA based immune assays are requested to cover the cost of purchasing fluorescently tagged antibodies for the purposes of immune-phenotyping inflammatory cells.

Animals ($45,000/year) Funds are requested to purchase ~160 each- SCID ($68), RAG ($124), young BalbC ($25), aged BalbC ($20), young B6 ($20) and aged B6 ($18) mice at the indicated prices per mouse. In addition, funds are requested to purchase ~60 golden Syrian hamsters ($43). These monies are essential for evaluating drug efficacy across hosts of differing susceptibilities to lethal infection, and to test drug efficacy in at least two animal species.

Other Expenses:

Animal per diem ($7,800/year) SCID, RAG and the young BalbC/B6 animals will be purchased and housed in UNC animal facilities for ~30 days prior to the start of experiments (5 animals per cage x 30 days x 0.65 per

cage). The aged BalbC/B6 animals will be purchased and housed in UNC animal facilities for ~90 days prior to the start of experiments (5 animals per cage x 90 days x 0.65 per cage).

Deep Sequencing ($10,000/year) The ExoN mutator phenotype results in high mutation rates which must be accessed by ultra deep sequencing methods like RNAseq, including informatics support to analyze the data. This also includes funds for supplies to generate amplicon library and to prepare the library for sequencing. As such, we anticipate significant sequencing costs over the duration of this proposal.

Maintenance Contracts ($5,000/year) Several instruments in the Baric Laboratory that will be used in these studies (4deg centrifuge, CO2-incubators, microscopes, BSL3 autoclaves) require service contracts for regular maintenance and repairs when needed. These are sophisticated instruments, so the repairs require specialists with appropriate tools and particular replacement parts. A fraction of these costs are included here.

Histology ($5,000/year) Histology slides from paraformaldehyde fixed tissues are prepared on a fee for service basis at UNC Chapel Hill. Given the large number of tissues to be analyzed each year, we are requesting funds to cover this tissue/slide preparation and staining costs.

Flow Cytometry ($8,000/year) UNC-Chapel Hill provides a core facility with advanced analytical cytometers that can resolve >6 colors at a time, which is needed when delineating subsets of inflammatory cells following infection.

Publication costs ($2,000/year) Funds are requested to cover the publication of manuscripts.

Shipping ($621/year) Funds are requested to cover the costs of shipping samples/viruses to the laboratory for analysis over the course of the proposal.

(b)(6) (b)(3):7 U S C § 8401

A. COMPONENT COVER PAGE

Project Title: Project 3.2 Novel Therapeutic Strategies Targeting Re-emerging Alphaviruses

Component Project Lead Information:

(b)(6) (b)(3) 7 U S C § 8401

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The goal of this project includes identification of novel small molecules capable of inhibiting replication of diverse members of the Alphavirus genus. Alphaviruses are arthropod-transmitted RNA viruses comprising seven antigenic complexes that include multiple Biodefense Category B and C priority pathogens. Alphaviruses are broadly comprised of geographically derived clades: New World [e.g. Eastern (EEEV), Venezuelan (VEEV), and Western Equine Encephalitis (WEEV) viruses) and Old World [e g. Chikungunya (CHIKV), Ross River (RRV), Semliki Forest (SFV), and Sindbis (SINV) viruses]. Two distinct pathologies are manifest during Alphavirus infection. Neurological disease including encephalitis is primarily associated with New World species and can present high mortality rates especially in hosts with weakened or immature immune systems as well as the young and aged populations. Arthralgia and inflammatory syndromes are typically associated with Old World species and while these are uncommonly fatal they can elicit incapacitating effects that persist long after viral clearance. Importantly, CHIKV is currently undergoing a severe re-emergence in areas around the Indian Ocean and Caribbean, an event that has involved evolutionary adaptation allowing inter-host transmission via mosquito species present in North America Currently no FDA approved vaccines or antiviral therapeutics are available to prevent Alphavirus infection or treat Alphavirus-associated disease Importantly, Alphavirus genomes mutate rapidly, greatly facilitating spontaneous changes in their host and vector ranges and virulence, and escape from prior immunity. We have found that the nucleoside analog Ribavirin inhibits CHIKV vRNA synthesis and replication, demonstrating that nucleoside and nucleotide analogs may represent viable therapeutic agents against Alphavirus disease Since the target of this class of inhibitors, namely RNA- dependent RNA polymerase (RnRp) activity, is well conserved among the Alphaviruses, compounds that impact these enzymes should target multiple species and perhaps other RNA virus clades such as Flaviviruses (Project 3), Coronaviruses (Project 2), and Influenza (Project 4) In light of this, experiments outlined in our proposal will utilize an established Alphavirus screening platform to examine a large, previously unexplored chemical library, heavily occupied by nucleoside and nucleotide analogs, by evaluating in vitro replication of two clinically relevant human Alphaviruses namely CHIKV (Old World) and VEEV (New World). This assay has been used to screen a compound library against VEEV and identified >100 that are active against VEEV Subsequent work will involve validation and mechanistic characterization of these efficacious compounds as well as additional ones identified in our primary HTS using unique molecular libraries Our goal is the identification of lead molecules for further in vivo evaluation using both murine and nonhuman primate models of infection. Parallel screening against multiple virus families using the same libraries by other members of this program will dramatically increase the likelihood of identifying antiviral compounds that are efficacious against a broad spectrum of agents In order to develop drug candidates that exhibit antiviral activity against multiple members of the Alphavirus genus we propose the following specific aims:

Aim 1: Employ a validated HTS primary assay to screen novel drug libraries for antiviral compounds that specifically block Alphavirus replication.
Rationale: Southern Research (SR) has developed and validated cell-based, high throughput assays for inhibitors of VEEV and CHIKV induced cytotoxicity Initial use of this assay has already identified several compounds with antiviral activity against VEEV Therefore, these assays will be employed to screen novel libraries of drugs that have not previously been screened against human pathogens including Alphaviruses.
Strategy: A CPE based assay will be used as a primary screen for antiviral compounds with activity against the Alphaviruses VEEV and CHIKV. Following these initial screens, "hits" will be evaluated in dose response and cytotoxicity assays to determine compound-specific EC50 , CC50 , and selective indices.

Aim 2: Validate and characterize antiviral activity and off-target effects.
Rationale: Hit compounds identified in the primary screen could potentially affect any stage of virus replication; therefore, we will characterize the anti-Alphavirus compounds with regard to efficacy and mechanism of action.
Strategy: We will use a variety of secondary assays to identify 1) breadth of anti-Alphavirus activity (test multiple Alphavirus species); 2) cell type-specificity (biologically relevant cells), 3) targets of antiviral compounds, and 4) ease of developing resistance phenotypes. Priority will be given to hits that are efficacious against many Alphaviruses and in multiple cell types, and do not affect virus entry or egress, nor activate IFN.

Aim 3:Chemical optimization and determination of in vivo efficacy of lead compounds
Rationale Our secondary assay characterization is expected to identify multiple compounds that specifically inhibit replication of diverse members of the Alphavirus genus Chemical optimization of effective scaffolds should generate compounds with greater efficacy, selectivity and bioavailability.
Strategy: Hit compounds identified and characterized above will be triaged by the Medicinal Chemistry and Lead Development Core. Compounds with appropriate activity and pharmacokinetic properties will be evaluated using CHIKV and VEEV models of acute and persistent infection and disease

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

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B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: Project 3 (b)(6), (b)(3) 7 B4.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

The plans for Year 3 in Project 3 can be found under the information for Project 3.1.

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

The accomplishments for Yr2 in Project 3 can be found under the information for Project 3.1.

B.4 (Project 3 (b)(6) (b)(3) 7 pdf)

B.4 WHAT OPPORTU[illegible]OR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

One Postdoctoral Fellow is active in the project. At UNC, Individual development plans (IDPs) are generated on an annual basis. They are used for defining key objectives and goals for progress and for review on at least an annual basis. For this project, the IDPs will review specific goals relevant to the project. For postdoctoral fellows, these assist in analysis of progress in projects; in addition they help in career development. For IDPs, both biosketches and CVs are appended, so that it is possible to use these as learning tools.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

C.5.a Other products

NOTHING TO REPORT

C.5.b Resource sharing

NOTHING TO REPORT

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable